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THE ROLE OF SEROTONIN RELEASE IN THE EFFECT OF
RESERPINE ON THE CENTRAL NERVOUS SYSTEM AND ON
THE THYROID GLAND

by

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A THESIS

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ABSTRACT

The inhibiting effects of reserpine on the central nervous system and on thyroid function were studied in an attempt to define the role of serotonin release in these effects.

It was found that methyldopa and reserpine depressed the spontaneous activity of rats. Associated with this depression which appeared to be a central effect was a decrease in the concentration of serotonin in the brain and thyroid gland. When methyldopa was administered prior to reserpine a slight antagonism of the effects of reserpine on these parameters was noted. The spontaneous activity of animals treated with reserpine was not related to the concentration of serotonin in the brain after the administration of reserpine. It was concluded that serotonin release is not the cause of reserpine-induced sedation.

Reserpine was found to depress thyroid function in rats as indicated by the measurement of radioiodine uptake. This effect of reserpine was partially dependent on the dose employed, and the time between administration of the drug, the radioiodine, and thyroid uptake analysis. The effect of reserpine on thyroid function was effectively blocked by hypophysectomy, and pretreatment with either dibenzylamine or methyldopa.

Reserpine, methyldopa, and guanethidine were seen to lower the concentration of serotonin in the thyroid gland. Treatment of animals with methyldopa followed by reserpine caused little immediate effect on the thyroidal serotonin depleting actions of the reserpine.

It was postulated that the thyroid depressant action of reserpine was the result of free brain serotonin activating the release of cortico-

tropin (ACTH) resulting in a decrease in thyrotropin (TSH) secretion.

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INTRODUCTION
AND LITERATURE
SURVEY

I THE ROLE OF SEROTONIN IN THE CENTRAL DEPRESSANT ACTION OF RESERPINE

Discovery of the presence of serotonin (5-hydroxytryptamine, 5-HT) in the mammalian brain by Amin et al in 1953 (1) and Twarog and Page in the same year (2) precipitated a very extensive world-wide pursuit into possibilities that the substance may have a role in brain function, perhaps as a chemical neurotransmitter(3,4). Support for this hypothesis was provided by subsequent work with drugs that affect the physiological disposition, storage, binding and pharmacophysiological actions of serotonin. Ironically enough, ten years have elapsed since the initial suggestion that serotonin may act as a neurotransmitter and despite the voluminous material available on the subject, little can be said without equivocation about a role for serotonin in chemical neurotransmission. Although serotonin passes most of the requirements for a neurotransmitter set forth by Brodie and Shore (5), it remains to be shown conclusively that serotonin does in fact participate in mental or psychopathological processes (4,6) as a neurotransmitter or otherwise. Most of the evidence for the hypothesis that serotonin does have an as yet undefined function in the brain has arisen from pharmacological and biochemical studies with drugs such as barbiturates, reserpine, lysergic acid diethylamide, chlorpromazine, and more recently, reserpine analogues and derivatives of tyrosine.

Thus, it was shown by Shore et al (7) that serotonin (20mg/kg) potentiated the hypnotic action of hexobarbital (100mg/kg) in mice. This effect was reduced by prior administration of 10mg/kg of lysergic acid diethylamide, a serotonin antagonist (8) which itself has a central effect in man resembling the mental disturbances seen in schizophrenia (9). It was also shown that reserpine potentiated the hypnotic effects of hexobarbital and ethanol in mice by a central action (10) and that this potentiating

action of reserpine was antagonized by lysergic acid diethylamide (11). These latter experiments suggested the possibility that some actions of reserpine might be mediated through serotonin release. It was found (11) that single doses of reserpine (3mg/kg) to dogs resulted in a marked increase in urinary excretion of 5-hydroxyindoleacetic acid (one of the end products of serotonin metabolism) which was apparent for eight hours or more. Similar treatment in the same animals the next day showed no such rise, indicating that the initial dose of the alkaloid resulted in maximal depletion of "available" serotonin depots. That a change in brain serotonin levels occurs after administration of reserpine was shown by Brodie et al (12) whereupon the serotonin content of the brain (species not stated) normally about 0.55µg/g, declined by about 90% within four hours after a single intravenous injection of 5mg/kg of reserpine. The low level persisted for twenty-four hours then rose slowly to normal in about seven days. These data were also reported by Hess et al (13). Doses of reserpine as low as 0.1mg/kg reportedly had an appreciable effect on brain serotonin levels.

That these effects of reserpine were not due to the drug itself but due to the free serotonin released by it (The "Brodie Hypothesis") was concluded from several experiments:

- 1) Reserpine given intravenously to rabbits enters the brain rapidly in relatively high concentration and thereafter rapidly disappears. The pharmacologic effects and loss in brain serotonin are of much longer duration (13).
- 2) The phenomenon is not entirely dose dependent (12) and in vitro studies with blood platelets showed that the release of serotonin is not stoichiometric with the amount of reserpine added to an incubate (14). Doses of reserpine ranging from 0.3µg/ml to 8µg/ml were equally effective in serotonin releasing potency.

- 3) Small amounts of serotonin entered the brain of mice given a large intraperitoneal dose of the substance. The animals displayed reserpine-like effects (15).
- 4) Serotonin was shown to depress spontaneous activity of rats and to produce a significant block of a conditioned response (pole climbing at the sound of a buzzer). The conditioned response was blocked by lysergic acid diethylamide (16).
- 5) The EEG sleep pattern seen with reserpine and serotonin (and chlorpromazine) was similar, with drowsiness and indifference, depression of the electrographic arousal reaction (evoked by electrical stimulation of the mid-brain reticular formation) and cortical excitability, and other effects (17).
- 6) When estrus rats were administered a large dose of the serotonin precursor 5-hydroxytryptophan (100mg/kg) the uterus took up considerable amounts of serotonin (ca. 2.5µg/g), apparently from the blood since the uterus normally contains no serotonin (18). In spite of this apparently bound (stored) serotonin present in a high concentration in the tissue, no contractile activity was seen when the uterus was suspended in Locke's solution. However, if a small amount of serotonin was added (.01µg/ml), a strong contraction resulted. Thus, free but not bound serotonin was found to be pharmacologically active in this preparation (19).
- 7) One of the actions of reserpine is increased intestinal motility and diarrhea due to free serotonin released by reserpine acting on the gastrointestinal tract (20).
- 8) After reserpine administration, the formation of serotonin is not impaired. Therefore, the storage sites which presumably synthesize the amine are unable to hold it and it is thus probably released in a free form (15).

Thus, the "Brodie Hypothesis" states that free serotonin released from within the brain by reserpine represents the balance between its rate of synthesis and destruction and thus floods specific sites in the brain resulting in central depression (14).

Since the original formulation of this hypothesis by Brodie and his colleagues, there have been several more recent reports published offering both support and criticism of the theory. Perhaps one of the more notable supportive findings is that of Garattini and Valzelli (21) and Sulser and Brodie (22) that the depressant action of reserpine occurs only in animals whose serotonin levels show a decline. Thus, in cold-stressed animals, reserpine was seen to lower brain norepinephrine levels (22) but not serotonin levels, and sedation was not seen (21,22). Although the physiological significance of these findings may be questioned since a cold-stressed animal can hardly be equated in responsiveness to drugs as one in a normal environment, nonetheless under the conditions of these experiments, reserpine-induced sedation was linked with serotonin release.

That reserpine also releases norepinephrine from brain (23,24,25) as well as other tissues has led certain investigators to speculate that reserpine-induced sedation is due to brain norepinephrine loss. Thus, Kärki and Paasonen employed the alkaloid raunescine and found that it lowered brain norepinephrine in rats but had no effect on brain serotonin and exerted a sedative effect (28). Pletscher and his colleagues (29) have supported this idea from studies with two benzoquinolizine derivatives Ro4-1284 and Ro4-1398 in mice. Their experiments showed that the former agent was more potent in releasing brain norepinephrine than the latter and no difference in serotonin releasing properties between the two agents was seen. Since Ro4-1284 was found to be a "stronger"

tranquilizer than its analog, these workers concluded that (the sedation) "may possibly be related to the depression of norepinephrine but probably not to that of serotonin". Brodie's group has strongly contested these arguments (30) and has presented direct evidence that none of the previously mentioned drugs including reserpine selectively release either serotonin or norepinephrine. In addition they have shown that the semi-synthetic reserpine analog Su-5171 can selectively release brain norepinephrine without accompanying sedation both in rabbits and rats.

Further support for the role of serotonin in reserpine-induced central depression is given from several reports that pretreatment of animals with iproniazid and then reserpine resulted in a prevention of serotonin and norepinephrine release by reserpine and marked central stimulation in these animals (31,32,33,34). This is apparently a mechanism whereby the amines are actually released by reserpine but are not metabolized by monoamine oxidase (35). In any event, reserpine is without sedating properties unless its amine-releasing properties are unhindered. Perhaps the exact mechanism is too complex to be attributed to a single component, as Dr. Vogt has stated (36): "...We have no doubt that reserpine does release HT^{*} from its site of storage in the brain, and we have equally no doubt that the same holds for other amines, the sympathomimetic amines. These two amines are selected in our minds only for the very fortuitous reason that these are the only two which have ever been investigated. I wonder whether it is not possible that there is no real correlation between the symptoms produced by reserpine and the disappearance of these two amines. Is this not possibly a model experiment showing that there may be other more important basic substances in the brain cells which are also released into the circulation, but which are unknown to us, and the concentration of which, perhaps, would correlate

* Serotonin

better with the action of reserpine than the loss or accumulation of HT?..."

The action of reserpine in man and animal is not only concerned with amine release, although perhaps this parameter of its action has received the most attention to date. Despite all of the work done with the drug, none of the findings about its pharmacodynamics are completely clear and not open to equivocation. The brain-amine problem has been adequately summed up recently by Broule and Costa in a recent paper entitled "Some Current Views on Brain Monoamines"(37): "At first the title of this talk "Review of Actual Knowledge in Brain Monoamines" somewhat puzzled us. However, our friends in Switzerland informed us that the word "actual" in French does not mean quite the same thing as "verite" in English, but only means "present" or "current"; and so there really was no need for us to hand in a blank manuscript!...."

11 THE ROLE OF SEROTONIN IN THE THYROID DEPRESSANT ACTION OF RESERPINE

Investigation into the thyroid depressant action of reserpine was largely initiated by the finding of Moncke (38) that chronic reserpine therapy produced normalization of the metabolic rate of hyperthyroid patients. In addition, reserpine was more effective than thiouracil in combating cardiac arrhythmias associated with this disease. Previous to this report Kuschke and Gruner (39) showed that reserpine interfered with the stimulating effects of exogenous thyroxin but not 2,4-dinitrophenol on oxygen consumption in rats, presumably by acting as a thyroxin antagonist. Although some reports have shown that reserpine produced no apparent change in metabolic rate (40,42) and thyroid I¹³¹ uptake in euthyroid patients or in experimental animals (43) after chronic treatment with reserpine, other communications have shown that reserpine is a thyroactive agent. Thus, De-Felice et al (41) showed that oxygen consumption

in reserpine treated euthyroid and hyperthyroid guinea pigs was reduced to levels found in hypothyroid animals. Hypothyroid animals did not respond significantly to the drug. Their analysis of these findings concluded that reserpine antagonizes the effect of thyroid hormone on oxygen consumption.

That reserpine is able to affect the thyroid in vitro was shown by Mayer et al (44) who found that reserpine added to incubating calf thyroid slices depressed the iodide trapping mechanism. Mayer et al concluded that this was a direct action of reserpine on this mechanism. More recently it has been reported (45) that reserpine in subcutaneous doses of 0.05 to $\mu\text{g}/\text{kg}/\text{day}$ in fowl resulted in depressed thyroxin release from the thyroid, inanition (exhaustion from lack of food) and weight loss. It was concluded that the depression of thyroid activity was secondary to the inanition caused by reserpine. Yamazaki et al (46) found that the "fractional rate of turnover" of exogenous radiothyroxin in mice was significantly decreased when high doses of reserpine (up to $25\mu\text{g}/\text{day}/\text{mouse}$) were given subcutaneously. Taylor (47) reported that the hypothermic action of reserpine shown by rats given $0.5\mu\text{g}/\text{kg}$ was due to decreased response of the thyroid gland to thyroid stimulating hormone. The findings of Ford et al (48) supported the earlier report by Mayer et al (44) that reserpine interferes with the organic binding of iodide. Ford and co-workers found that reserpine in a dose of $0.3\mu\text{g}/\text{kg}/\text{day}$ in rats had no effect on the uptake and degradation of triiodothyronine in the thyroid. Therefore it would seem that the drug acts at the level proposed by Mayer et al (44), and does not appear to interfere with iodide uptake once the iodide has been incorporated.

The question now arises as to whether or not this action of reserpine is mediated by an action on the hypothalamo-hypophyseal system or is due to a direct action of reserpine on the thyroid gland. In this regard there has been much interest in the action of reserpine on the pituitary-adrenal system. It was shown by Wells et al (49) that a single injection of reserpine in rats resulted in an acute hypersecretion of ACTH but no such increase was observed after daily administration of reserpine after a period of five to seven days. This was concluded to be the result of a central action and not due to a direct effect of reserpine on the adrenal cortex. Subsequently, Maickel et al (50) showed that reserpinized rats exhibited a biochemical response similar to that resulting from cold stress, namely depletion of adrenal ascorbic acid, increased plasma corticosterone, increased liver tryptophan pyrrolase activity, adrenal hypertrophy and free fatty acid release. ACTH hypersecretion was implicated as the mechanism in these effects since they were not seen in adrenalectomized or hypophysectomized rats. Westermann et al (51) have since shown that ACTH release by reserpine is related to blockade of brain monoamine storage since only in doses that lower the amine (serotonin and norepinephrine) stores by 50 percent or more is ACTH discharge elevated. Alpha-methyl-meta-tyrosine (which has no long-term effect on brain serotonin but depletes norepinephrine) had only a slight effect as compared to reserpine on the pituitary-adrenal system(51). Not until after reserpine was given in these animals and a drop in brain serotonin occurred was there any evidence of ACTH hypersecretion. With reference to the earlier effects mentioned that monoamine oxidase inhibitors prevent reserpine-induced serotonin release, Martel et al (52) have shown that similar pretreatment also prevents the sedation and pituitary-adrenal response to reserpine.

The possibility therefore exists that a portion, at least, of the antithyroid action of reserpine is mediated centrally since ACTH and physical and emotional stress are known to inhibit thyroid function (53,54). The suggestion will be made in a subsequent portion of this manuscript supporting a hypothesis that reserpine induced hypersecretion of ACTH is in part, at least, responsible for the depression of thyroid activity after the drug. That reserpine may be acting peripherally, i.e. on the thyroid by an indirect means has been suggested recently by Williams and Coker (55). These workers attributed the inhibition of thyroid by reserpine to serotonin release, presumably from peripheral sources. It is quite likely that both a central and peripheral mechanism may be present in the drug-induced syndrome.

111 METHYLDOPA

Methyldopa was first described by Sourkes (56) as an in vitro inhibitor of dihydroxyphenylalanine (dopa) decarboxylase. Smith (57) presented evidence that methyldopa was an effective in vitro and in vivo inhibitor of 5-hydroxytryptophan (5-HTP) decarboxylase. In addition, reduced serotonin levels were seen in the mouse brain and intestine after administration of large doses of the drug. Hess et al (58) have shown that methyldopa lowers the level of serotonin, norepinephrine and dopamine in the guinea pig by a reserpine-like release mechanism and probably not by virtue of its inhibiting action on the decarboxylating enzymes which form serotonin and dopamine from their amino acid precursors. Increasing amounts of evidence suggest that the decarboxylases which are necessary in the formation of serotonin and catecholamines are one and the same (59-62). It might be expected therefore that if the drug were to lower the amount of serotonin in tissues by inhibition of 5-HTP decarboxylase a similar situation would result for the catecholamine

levels in tissue. The report by Hess et al (58) has shown that animals pre-treated with α -methyl amino acids are capable of forming norepinephrine. This observation would indicate that in vivo inhibition of dopa- and 5-HTP decarboxylase plays only a small role, if any, on the amine-depleting effects of methyldopa. In addition, Hess et al (58) presented evidence that depression of norepinephrine levels in the guinea pig heart was evident for several days after the enzymatic effects of methyldopa were no longer apparent. It might be concluded, therefore, that with regard to tissue biogenic amine levels methyldopa acts in a manner similar to reserpine. This concept has been supported by Schanberg and Giarman (63) who have shown that methyldopa causes, like reserpine, an increase in the amount of free serotonin in the brain after a single dose.

That methyldopa is an in vivo inhibitor of 5-HTP and dopa decarboxylase has been shown by several workers. In the report published by Smith (57) methyldopa was seen to inhibit the activity of 5-HTP decarboxylase in the brain and small intestine of the mouse. Westermann et al (59) showed that the drug prevented the bronchoconstricting action of administered exogenous 5-HTP which is normally mediated by its decarboxylated product, serotonin. Dengler and Reichel (64,65) gave evidence that methyldopa inhibited the pharmacologic actions of administered dopa which are normally mediated by the catecholamines obtained from its decarboxylation.

Methyldopa has been examined clinically as an antihypertensive agent in man (66-69). The original purpose of this clinical work was based on the hypothesis that methyldopa inhibited the formation of pressor amines by virtue of its in vivo antidecarboxylase properties with accompanying depletion of catecholamines from central and peripheral sympathetic nerve networks. Experiments have shown that the drug is an

effective antihypertensive agent (66-69) but its hypotensive properties are apparently unrelated to the previously documented actions as an antidecarboxylase (70). Goldberg et al (70) have shown that the inhibiting effect on decarboxylation was apparent immediately after an intravenous dose of the drug. This antidecarboxylase effect was no longer apparent or was disappearing at the time that the hypotensive action was beginning to appear. It may be argued, however, that the hypotensive effect of the drug may be initially masked by the increased amount of circulating pressor amines which would be made available (released) by methyldopa. Such a masking effect has been illustrated with the effect of reserpine on the dog's blood pressure (71). In addition, the lack of correlation of antihypertensive effect of methyldopa with in vivo antidecarboxylase action may be due to the fact that methyldopa is itself a substrate for the decarboxylase enzymes which it inhibits; i.e. it acts competitively (56,72-74). The resulting alpha-methylated catecholamines (74) could exert a pressor effect which would obviously antagonize or mask any apparent antihypertensive action. Suggestions have been made that the antihypertensive action of methyldopa is, in man, due to a decreased cardiac output without compensatory vasoconstriction (69), and/or due to decreased systemic arteriolar tone without change in cardiac output (75). Apparently the sedative effects of the drug (57) are not a part of the antihypertensive effects (69).

STATEMENT OF THE PROBLEM

1 The Role of Serotonin in the Central Depressant Action of Reserpine

Since Brodie's theory (see page 2) implies that free serotonin from within the brain mediates the depressant action of reserpine it was decided to study the effect of pretreatment of animals with an inhibitor of 5-hydroxytryptophan decarboxylase, the enzyme which decarboxylates 5-hydroxytryptophan to form serotonin (76), before the administration of reserpine. In so doing the animals would have a decreased rate of serotonin synthesis while under the influence of such treatment. The anticipated achievement of such maneuver would be to slow down the rate of synthesis of serotonin in the reserpinized animals. Thus, the so-called "free brain serotonin" level after reserpine should be decreased. If this did occur, then the action of reserpine, according to Brodie's hypothesis, would be antagonized or somewhat decreased.

If the central actions of reserpine may be attributed primarily to a loss of serotonin from functional storage sites rather than to the effect proposed by Brodie, then a decreased level of serotonin in the brain at the time of reserpine administration should conceivably result in a decreased effect of reserpine on the central nervous system.

In addition, it was decided to be of interest to quantitate the duration of depression of animal activity after a single dose of reserpine and for comparsion, methyldopa in an attempt to correlate the degree of activity in the animals with brain serotonin levels. It has been stated that such a correlation does not exist in reserpinized animals (5) and animal activity appears normal a few days after the drug, inasmuch as the level of serotonin in the brain remains depressed for up to one week after a dose of the drug (13). It is known that animals given a dose of reserpine become aggressive and hostile and may on occasion bite at one's gloved hand when it is inserted into a cage containing a reserpinized rat. This phase of

hyperexcitability after reserpine has received little attention and was therefore chosen as an area worthy of investigation in this project. In this portion of the project the spontaneous activity of rats given a single dose of reserpine was measured for up to sixty hours after a dose of the drug. This provided a quantitative measurement of the recovery of the animals after a single dose since previous observations on this phenomenon have been purely of a subjective nature.

II The Role of Serotonin in the Thyroid Depressant Action of Reserpine

Previous observations that the thyroid gland is relatively rich in serotonin content in some species (77,78) and that it contains catecholamines (79) and monoamine oxidase (80) aroused the speculation that a mechanism for the thyroid depressant action of reserpine might be mediated through thyroidal serotonin release. In other words, the biochemical make-up of the thyroid was not unlike that of the brain with regard to its biogenic amine content. Most of the investigators who have reported that reserpine has no effect on thyroid function were using chronic drug administration. The report by Shore et al (11) showed that after the second of two successive doses of reserpine administered twenty-four hours apart in the dog there was no increase in urinary 5-hydroxyindoleacetic acid excretion. This raised the possibility that thyroid depression after reserpine may be seen only during the initial "avalanche" release of serotonin following the first dose of reserpine, a concept not unlike that proposed by Brodie for the central depressant action of the drug. It therefore appeared logical to investigate the possibility that reserpine acts in or on the thyroid in a manner analogous to that known to occur in the brain, in other words that the action is an indirect one and is brought about by the release of serotonin. Whether or not this amine-release mechanism is a direct

consequence of free intrathyroidal amines or is a consequence of the action of reserpine on the pituitary-adrenal axis was chosen as an area of investigation in an attempt to provide some evidence for either or both mechanisms in the total effect seen on thyroid function after a dose of reserpine.

METHODS AND MATERIALS

1 THE ROLE OF SEROTONIN IN THE CENTRAL DEPRESSANT ACTION OF RESERPINE

1 Animals

Male Sprague-Dawley rats of approximate average weight 160 grams were used in all experiments. The animals were maintained on Purina Fox Chow and tap water with the exception that the animals were starved for twelve hours before use. In some long-term experiments with reserpine food was given twenty-four hours after the drug to prevent inanition from influencing the experiments.

2 Drugs

All drugs used were administered by the intraperitoneal route. Reserpine was administered in a dose of 2mg/kg body weight. It was prepared as a 200 mg % solution in the following vehicle (81):

Reserpine	100 mg
Benzyl Alcohol	1 ml
Citric Acid (Anhydrous)	125 mg
Polysorbate - 80	5 ml
Water, to make	50 ml

This solution was maintained in the refrigerator in an amber bottle and was freshly prepared each month. Methyldopa* was given in a dose of 100mg/kg body weight in the following vehicle which was found suitable for solubilizing the drug in a 5% (gm/100ml) concentration:

Methyldopa	500 mg
Benzyl Alcohol	1 ml
Polysorbate - 80	2 ml
Propylene Glycol	4 ml
Water, to make	10 ml

* Aldomet^R (Merck Sharp and Dohme)

Warming the mixture under a running warm water faucet facilitated solution of the drug. This solution was prepared daily immediately before use since the drug was found to precipitate from solution within a few hours. Control animals were given appropriate volumes of the individual vehicles.

3 Animal Activity Measurements

The animals were gently placed in a six-photocell small animal actimeter* at the required time interval after administration of the drugs. Each animal was used once only in these experiments. Activity under the influence of the drugs was measured in this device only in the daylight hours in a corner of the laboratory removed from extraneous noise and direct lighting#. This served to reduce the influence of the direct light on the activity of the animal and allowed the actimeter to function correctly. In each experiment the test animals were kept in the device for a ten minute period. Activity was expressed as the number of counts registered per minute. Animals which had received either of the drugs or the solvents were immediately decapitated for the second part of the experiment which was the estimation of serotonin in the brain and thyroid gland.

4 Estimation of Serotonin

Serotonin was estimated fluorometrically (82) after extraction from tissues with the solvent system previously described for extraction of norepinephrine by Shore and Olin (83), except that some modifications were made for convenience. Accurately weighed tissue samples were homogenized in sufficient 0.01N hydrochloric acid to give a total volume of 3.0 ml.

* Woodard Research Corp., Herndon, Va., U.S.A.

The device did not respond satisfactorily unless placed away from direct overhead lighting

The homogenate was transferred quantitatively to a 40 ml stoppered centrifuge tube containing 2 grams of sodium chloride. Portions of the homogenate adhering to the glass wall of the tissue grinder were washed into the centrifuge tube with 15.0 ml of n-butanol purified as described by Shore and Olin (83), in the following manner.

Normal butanol was shaken well with an equal volume of 1N sodium hydroxide. After phase separation the alkali layer was removed and the butanol was then shaken with an equal volume of 1N hydrochloric acid. Following removal of the acid layer, three similar washings were made with distilled and demineralized water. A final wash of the butanol was made with an equal volume of 0.01N hydrochloric acid. Excess acid in the butanol was removed by saturation of the butanol with sodium chloride.

The acid homogenate butanol mixture was then shaken in a mechanical shaking device for fifteen minutes. A 10.0 ml aliquot of the clear supernatant butanol obtained after centrifugation for fifteen minutes at 2000 r.p.m. was added to another 40 ml stoppered centrifuge tube containing 20 ml of n-heptane and 3.0 ml of 0.1N hydrochloric acid. The heptane was purified by successive washing with 1N sodium hydroxide, 1N hydrochloric acid, and three washings with distilled water. The mixture was then shaken for five minutes and centrifuged as above. The heptane layer and any residual solid material remaining at the organic-aqueous interface was carefully removed by aspiration. A 1.0 ml aliquot of the resulting clear acid layer was transferred to a 1 cm² quartz cell and mixed with 0.3 ml of 12N hydrochloric acid. The concentration of serotonin was then determined fluorometrically as described by Bogdanski et al (82) using the Aminco-Bowman Spectrophotofluorometer with the following instrument settings :

Activation wavelength	295 mμ
Fluorescence wavelength	540 mμ
Slit arrangement	#5
Phototube (visible sensitive)	1P21
Photometer sensitivity	0

The settings of wavelength were not calibrated.

It was found possible to recover 92.5% when serotonin (Serotonin Creatinine Sulfate; Nutritional Biochemicals) was carried through the entire extraction procedure. When serotonin was added to homogenates the average recovery was 88.5% (see APPENDIX). That this solvent system will extract serotonin from tissue has been previously verified (84). Agreement of activation and fluorescence spectra between authentic serotonin and tissue extracts was taken as additional evidence that the substance being estimated was in fact, serotonin. Norepinephrine and dopamine did not interfere in the analysis when added to homogenates in amounts up to 2 μg. Concentrations above this amount would not occur and so were not examined. The concentration of serotonin in tissue was reported according to the following equation :

$$\text{Serotonin}^* \text{ Concentration} = \frac{(\text{Photometer \% Transmission} \times \text{Meter Multiplier}) - \text{Blank}}{\text{Fresh Tissue Weight in Grams}}$$

Standard solutions were prepared daily to check the performance of the instrument and when necessary corrections were made. The level of serotonin in the normal rat brain was found to be 0.54 ± 0.01 μg/gram (mean $\pm$ S.E. of 16 determinations). The rat thyroid was found to contain $3.55 \pm .05$ μg/g (mean $\pm$ S.E. of 4 determinations on 32 thyroid glands pooled into groups of eight). These values are in excellent agreement with data reported in the literature (77,85).

* The data in the tables presents figures for serotonin concentration which are given as above. In graphs the serotonin concentration is reported as μg/g and also as percentage of that established from normal animals.

II THE ROLE OF SEROTONIN IN THE THYROID DEPRESSANT ACTION OF RESERPINE

1 Animals

Male Sprague-Dawley rats maintained as detailed above and fasted for twelve hours prior to use were used in these experiments. Adrenalectomy was done through an incision in the back under ether anesthesia. Adrenalectomized animals were maintained for three days prior to use on the regular diet with 1% sodium chloride added to the drinking water. Hypophysectomized animals of the same strain were obtained commercially* and were maintained on laboratory diet supplemented with fresh oranges, bread, and carrots.

2. Drugs

Methyldopa and reserpine were prepared and administered as described in the preceding section. Phenoxybenzamine hydrochloride (Dibenzylamine) was dissolved in the solvent used for methyldopa and was administered in a dose of 2mg/kg body weight. Guanethidine sulfate was administered in an aqueous dilution of commercially available ampuls of Ismelin^{R**} in a dose of 5mg/kg body weight. All drugs were administered intraperitoneally. Control animals received appropriate volumes of the vehicle applicable in each case.

3 Procedure for radioiodine uptake measurements

Radioiodine as NaI¹³¹ was administered intraperitoneally in a dose of 20µc/kg body weight. The animals were sacrificed at an appropriate time by diethylether over-anesthesia. The tracheal chain was removed by ventral incision into the neck region. Both lobes of the thyroid gland were removed quickly and weighed immediately after removal of adhering adipose tissue. Care was taken to keep the operation as bloodless as possible to prevent contamination of the excised glands with blood. The excised glands were then refrigerated in separate petri dishes until used for radioactivity

* Hormone Assay Laboratories, Chicago, Ill., U.S.A.

** Ciba

assay. A sodium iodide (TI) crystal well-type scintillation detector was used for measurement of the thyroidal I^{131} taken up in the glands. Each gland pair was placed in the bottom of a test tube which was then placed into the well for counting. A three minute count was obtained in duplicate from each sample and averaged. In each case standard solutions were assayed at the same time to correct for loss in gland I^{131} activity due to natural radioactive decay. An accurately measured volume of the radioiodine solution injected was assayed for radioactivity. This measure enabled calculation of thyroidal I^{131} uptake on the basis of % recovery of the number of counts per minute injected into each animal. The pulses from the scintillation detector (crystal unit) were passed via a radiation analyzer (energy spectrometer) to a decade scaler. The analyzer was channelled to detect only the 638 KEV gamma radiation from the I^{131} . This procedure eliminated the background count error usually present and permitted the capture of most of the higher energy radiation from the I^{131} source. The instrument was checked for counting error from coincidence loss and was found to be responding in a linear fashion to the amount of radioactivity present. The following instrument parameters were used:

Scaler high voltage	2500 V
Spectrometer window	on
Window width	5V
Spectrometer high voltage	680 V
Pulse Height	638 KEV on Gain 4

All results were calculated as percent uptake of administered dose of I^{131} per mg thyroid gland weight in order to correct for small differences in the amount of I^{131} administered to each animal. The following equation will illustrate the method of calculation:

% of dose per mg :

$$\frac{\text{mean counts per min. of gland}}{\text{mean counts per min. of standard}} \times \frac{\text{vol. standard counted}^*}{\text{vol. of dose of I-131}} \dots\dots\dots$$

$$\dots\dots\dots \times \frac{1}{\text{gland weight (mg)}} \times 100.$$

* This fraction was reversed when the volume of injected dose was greater than the volume of standard solution counted.

In other words, the percentage uptake per mg gland tissue was calculated as a recovery of the number of counts per minute administered as outlined above. Constant counting geometry was observed throughout the experiment.

III STATISTICAL PROCEDURES

The statistical procedures, including calculations of standard deviation, standard error, significance tests, and regression correlations were done according to standard procedures (86).

RESULTS

I THE ROLE OF SEROTONIN IN THE CENTRAL DEPRESSANT ACTION OF RESERPINE

1. Studies on normal animals

The serotonin concentration in selected tissues and spontaneous activity were examined in untreated animals according to the procedure given in the section on methods. The data illustrated in Figure 1 and Table 1 indicate that the activity of the animals as measured in the actimeter was not related to body weight in the weight range studied. Although there was considerable variation in the weights of the animals it was possible to compare all of the results. The mean activity was found to be 38 counts per minute with a standard deviation of 11 counts per minute.

The mean of sixteen estimations of the concentration of serotonin in brain tissue was found to be 0.81 with a standard error of $\pm .02$ calculated as the net fluorometer % transmission divided by the fresh tissue weight in grams as previously outlined (page 20). When the individual figures were calculated on the basis of a standard curve obtained for serotonin the average figure obtained was 0.54 $\mu\text{g/g}$ fresh tissue. This value agrees well with data obtained by other workers using a similar fluorometric assay method for serotonin estimation (5,14,37).

The value for 13.4 ± 0.2 for the normal concentration of serotonin in the rat thyroid gland found by this method similarly corresponds to a concentration of 3.55 $\mu\text{g/g}$ fresh tissue. Paasonen (77) and Paasonen et al (78) have reported values of 3.7 $\mu\text{g/g}$ and 6.0 $\mu\text{g/g}$ for a concentration of serotonin in the thyroid gland of the male rat. These values were obtained using biological assay methods. The concentration of serotonin in the thyroid seems extraordinarily high compared with the amount of serotonin in the brain. Whether or not this level of serotonin in the thyroid gland is of physiological significance is not immediately apparent since little work has been reported

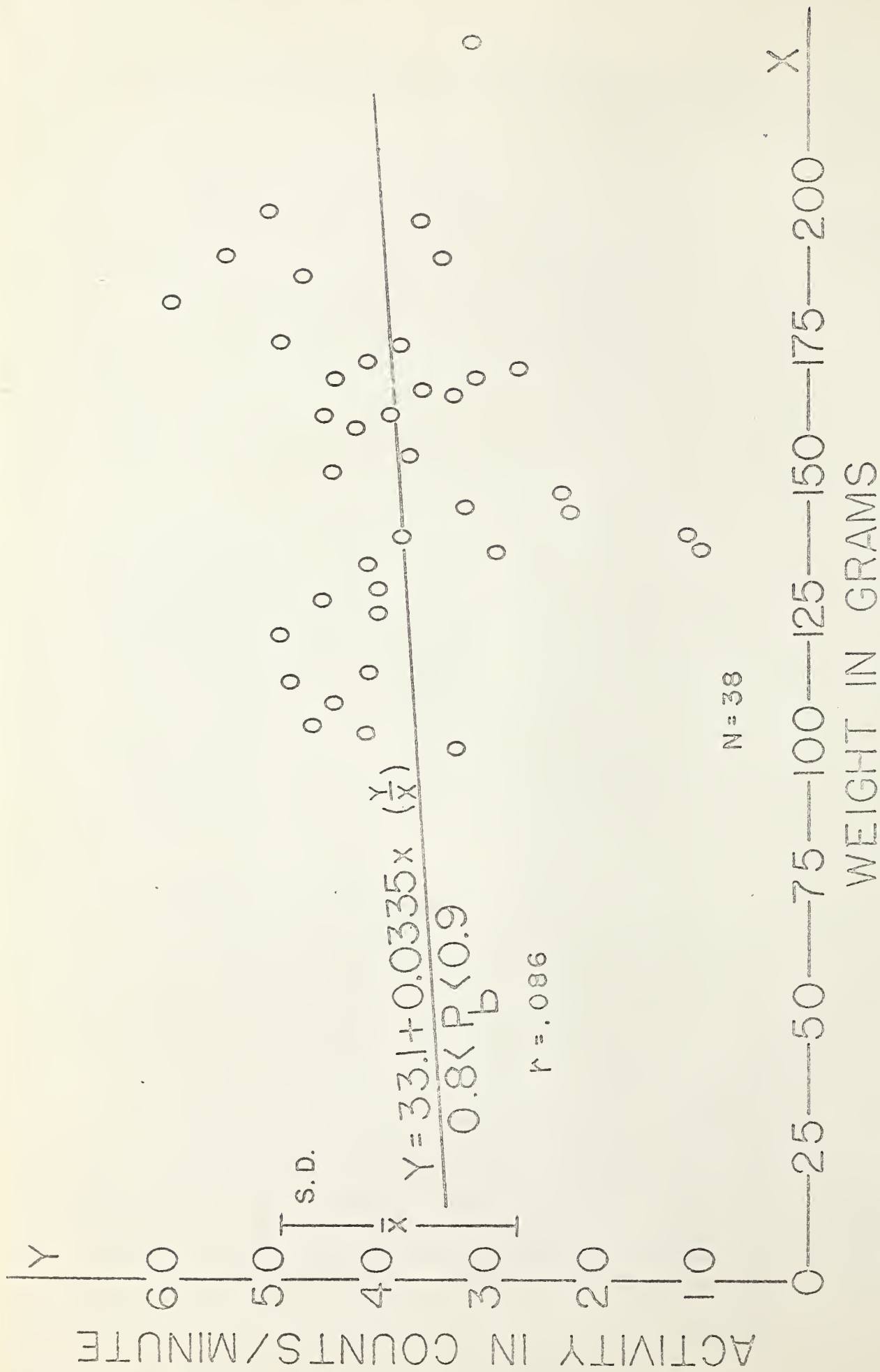


Figure 1: The Relationship of Spontaneous Activity of Male Rats to Body Weight.

The regression line of $y(\text{activity})$ on $x(\text{weight})$ has a Pearson's correlation coefficient of 0.086, indicating very little cause and effect relationship between body weight and activity. In addition, the regression coefficient 0.0335 (slope of the regression line) is not significantly different from zero ($0.8 < P < 0.9$).

TABLE I Spontaneous Activity and Tissue Serotonin Levels in Normal Male Rats

No.	Weight (g)	Activity c/m	Serotonin	
			Brain	Thyroid
1	103	46	0.81	13.4
2	135	29	0.78	
3	113	41	0.66	
4	107	44	0.91	
5	124	40	0.81	
6	133	41	0.79	
7	160	45	0.96	
8	138	38	0.85	
9	143	22	0.78	12.8
10	146	23	0.74	
11	128	45	0.82	
12	138	11	0.73	
13	136	10	0.86	
14	150	44	0.85	
15	164	33	0.72	
16	128	40	0.83	
17	167	31		13.9
18	181	59		
19	190	54		
20	158	42		
21	167	38		
22	170	44		
23	192	41		
24	160	34		
25	165	39		13.5
26	174	36		
27	144	49		
28	169	32		
29	153	27		
30	186	37		
31	230	47		
32	198	50		
Mean	155	38	0.81	13.4
S.D.		11	0.07	0.3
S.E.		2	0.02	0.2
% S.E.		5	2.2	1.3

Serotonin figures represent the net fluorometer % fluorescence reading divided by the fresh weight of brain (g), and thyroid from 8 pooled tissues. Normal values in ug/g are: Brain 0.54, Thyroid, 3.55.

on this aspect (77, 78).

2. The effect of reserpine on the spontaneous activity of rats and brain serotonin concentration.

A single intraperitoneal dose of 2mg/kg of reserpine caused a depression of spontaneous activity in the animals (Figure 2, Table II). This depression was not significantly different from the control animals at the two-hour interval ($0.4 < p < 0.5$) but was highly significant four hours after administration of the drug. A gradual recovery in spontaneous activity was seen to begin twelve hours after administration. The activity returned to normal within thirty-six hours. The period of hyperactivity seen at the forty-eight and sixty hour intervals differed slightly from normal activity ($0.02 < p < 0.05$). Some depression of spontaneous activity from that of normal animals was noted at two hours in animals treated with a proportional amount of the reserpine vehicle (Table III, Figure 2). This depression was not significant ($0.3 < p < 0.4$) and was probably due to the trauma of the injection. The reserpine vehicle was without effect on the concentration of serotonin in the brain (Table IV).

It would appear that the depressant action of reserpine is not related to the serotonin concentration in the brain per se. The data shown in Figure 3 and Table V would seem to support this conclusion. It can be seen from these data that the concentration of serotonin in the brain decreased in an approximately parallel fashion to the decrease in spontaneous activity. That there is no overall relationship between animal activity and brain serotonin levels after reserpine is supported by the finding that the animals showed almost complete recovery of activity after twenty-four hours but there was no comparable recovery of brain serotonin concentration. Furthermore, these data (Figure 3, Table V) indicate that the brain serotonin concentration remained maximally depressed for up to sixty hours following the administration of reserpine while the animals exhibited a certain degree

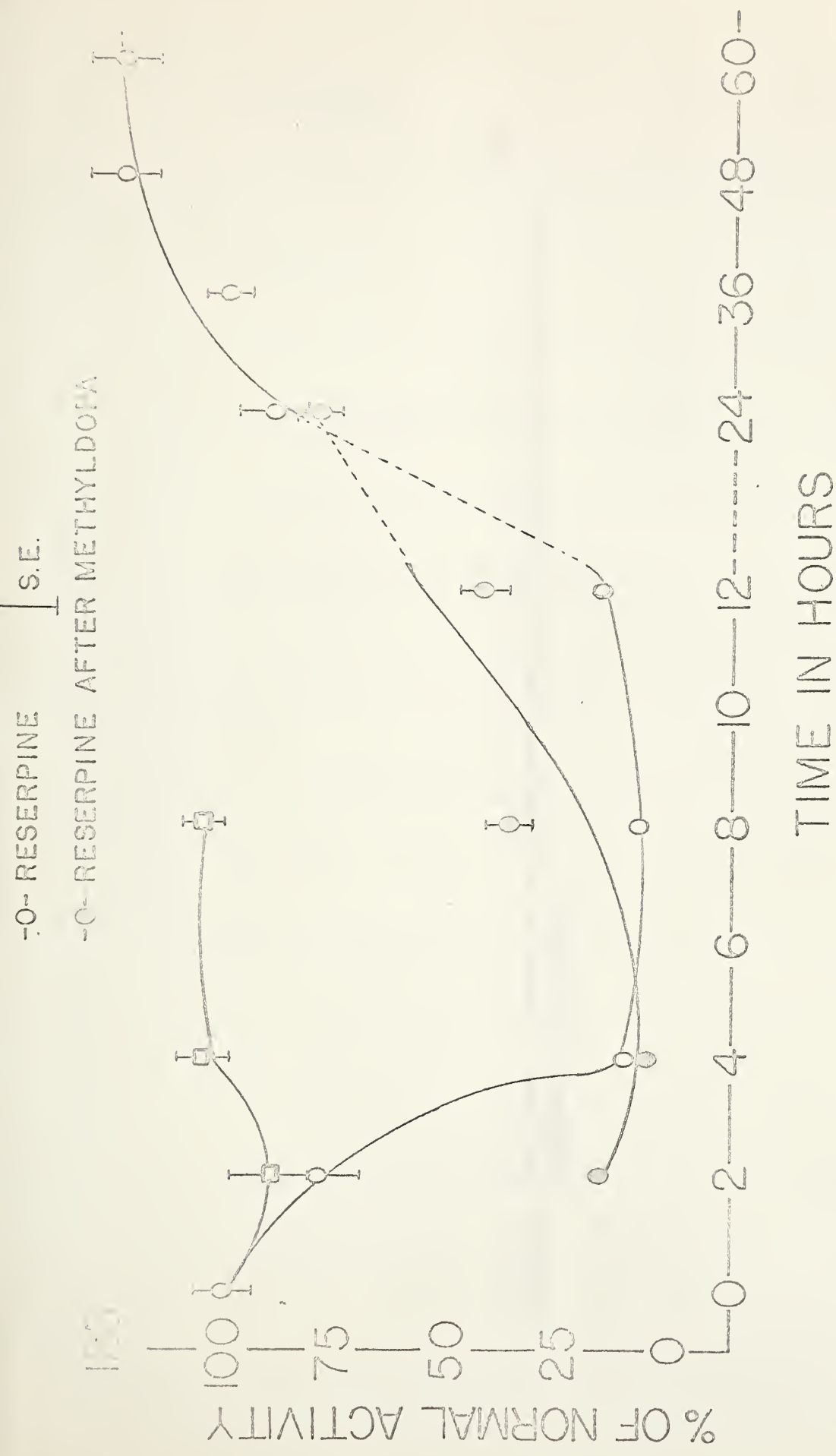


Figure 2: The Effect of Reserpine on Spontaneous Activity of the Male Rat With and Without
Methyldopa Pretreatment.

TABLE II The Effect of Reserpine (2 mg/kg, i.p.) on Spontaneous Activity of the Rat

No.	Time After Drug Administration									
	2 Hours	4 Hours	8 Hours	12 Hours	24 Hours	36 Hours	48 Hours	60 Hours		
1	44	3	0	3	42	48	40	37		
2	56	3	10	14	30	19	42	40		
3	32	4	0	2	32	48	60	53		
4	15	2	3	0	16	28	38	43		
5	24	2	3	9	25	35	48	61		
6	32	4	2	10	18	30	45	38		
7	34	7	4	3	31	50	37	57		
8	23	10	0	0	38	29	64	47		
9	22	4	3	8	45	36				
10	28	6	4	2	47	42				
11	20	5	0	12	17	38				
12	47	2	2	9	45	32				
13	41	2	1	10	27	48				
14	6	3	6	4	43	52				
15	27	3	2	0	29	37				
16	28	2	3	6	52	30				
Mean Counts/Min.	30	4	3	6	34	38	47	47		
S. D.	12	2	2	4	11	9	10	8		
S. E.	3	0.5	0.5	1	3	2	4	3		
% S.E.	11	13	18	20	9	6	8	7		
Mean Weight (g)	170	168	181	179	182	162	187	152		

Normal activity: 38 c/m (TABLE I)

TABLE III The Effect of Methyldopa and Reserpine Placebo (Controls) on Spontaneous Activity of the Rat

Time After Drug Administration						
No.	2 Hours		4 Hours		8 Hours	
	Methyldopa	Reserpine	Methyldopa	Reserpine	Methyldopa	Reserpine
1	15	25	18	30	32	39
2	13	28	26	31	34	54
3	12	32	40	54	31	42
4	34	26	34	44	42	40
5	12	40	15	39	30	34
6	26	38	30	40	28	33
7	12	34	31	37	50	38
8	18	51	45	44	50	36
Mean C/M	18	34	30	40	38	40
S. D.	8	8	10	7	9	6
S. E.	3	3	4	3	3	2
% S.E.	16	9	12	7	9	6
Mean Weight (g)	201	110	190	109	190	109

Normal Activity: 38 c/m (TABLE I.)

TABLE IV

The Effect of Reserpine Placebo (Controls) on Brain and Thyroid
Serotonin in the Male Rat

(The figures in each column refer to the net fluorometer % fluorescence
per gram fresh tissue)

No.	Time After Drug Administration					
	2 Hours		4 Hours		8 Hours	
	Brain	Thyroid	Brain	Thyroid	Brain	Thyroid
1	0.75		0.76		0.69	
2	0.84		0.84		0.69	
3	0.80		0.63		0.82	
4	0.77	13.6	0.89	12.6	1.01	13.8
5			0.67		0.83	
6			0.81		0.74	
7		13.5	0.82	13.7	0.78	12.8
8			0.79		0.80	
Mean	0.79	13.6	0.78	13.2	0.80	13.3
S. D.	.03		.06		.09	
S. E.	.02		.02		.04	
% S.E.	2.3		3.1		4.5	
Mean Weight (g)	160		100		109	

Normal Values are: Brain, 0.81; Thyroid, 13.4. (TABLE I)

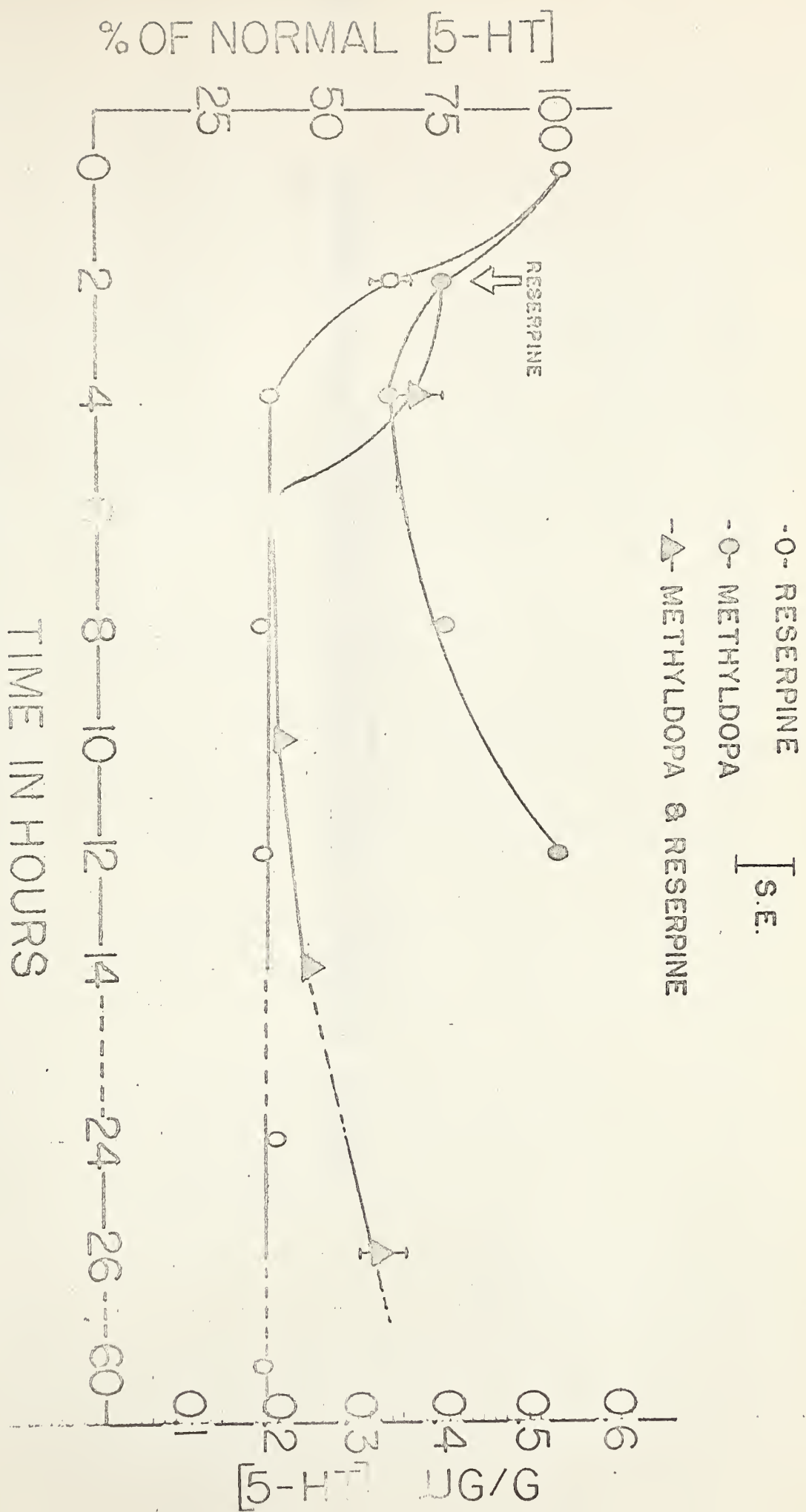


Figure 3: The Effect of Reserpine, Methyldopa, and Methyldopa Followed by Reserpine
on the Brain Serotonin Concentration in the Male Rat.

TABLE V The Effect of Reserpine (2 mg/kg, i.p.) on Brain Serotonin in the Rat

(The figures in each column refer to net fluorometer % fluorescence per gram fresh brain tissue)

Time After Drug Administration						
No.	2 Hours	4 Hours	8 Hours	12 Hours	24 Hours	60 Hours
1	0.71	0.29	0.33	0.26	0.32	0.16
2	0.76	0.32	0.30	0.31	0.29	0.26
3	0.48	0.30	0.30	0.25	0.31	0.29
4	0.61	0.28	0.29	0.28	0.31	0.30
5	0.64	0.30	0.27	0.29	0.25	0.34
6	0.68	0.35	0.24	0.25	0.29	0.27
7	0.33	0.27	0.29	0.25	0.26	0.31
8	0.43	0.24	0.21	0.25	0.31	0.21
9	0.45	0.36	0.30	0.32	0.27	
10	0.57	0.34	0.28	0.27	0.32	
11	0.43	0.32	0.28	0.28	0.36	
12	0.45	0.29	0.24	0.22	0.29	
13	0.34	0.25	0.33	0.30	0.29	
14	0.43	0.31	0.30	0.31	0.31	
15	0.32	0.30	0.31	0.29	0.30	
16	0.46	0.29	0.26	0.26	0.28	
Mean	0.51	0.30	0.28	0.27	0.30	0.27
S.D.	0.13	0.04	0.03	0.03	0.03	0.06
S.E.	0.03	0.01	0.009	0.008	0.008	0.02
S.E.	6.8	3.2	3.0	2.8	2.7	8.0
Mean Weight (g)	170	168	181	179	182	152

Value for normal % fluorescence/g fresh tissue is 0.81 (TABLE I), which represents a concentration of serotonin in the brain of 0.54 ug/g.

of hyperactivity. In view of the finding that there is no apparent correlation between activity and brain serotonin concentration in reserpine-treated rats it would seem hardly likely that Brodie's hypothesis (page 2) is valid. Since there was no detectable recovery of serotonin binding sites which would have been indicated by an increase in brain serotonin concentration at or during the time that the animals displayed a return of spontaneous activity to normal or hyperactive levels, there is no apparent cause and effect relationship between serotonin release and the depressant action of reserpine. It was originally proposed by Shore et al (11) that at the time of recovery from the sedative action of reserpine the serotonin binding sites were regaining their ability to "inactivate" the free serotonin released by reserpine by a binding process. The limiting factor in this argument against a causal relationship between serotonin release and reserpine-induced sedation is the validity of the photocell actimeter technique employed in measurement of spontaneous movements as an index of total central nervous system activity. In support of this technique, however, it may be argued that the approach is perhaps more objective in measurement of a "subjective" parameter, such as spontaneous activity, than simply observing an animal and applying various grades (eg. +, ++, +++ etc.) of activity based on subjective observations. In other words, human bias in measurement is largely eliminated by this technique.

Although the spontaneous activity of the reserpinized animals was normal after twenty-four hours following injection of the drug the persistence of other pharmacologic signs was noted. The animals did not immediately eat the food given them twenty-four hours after the reserpine, eyelid ptosis was reduced in degree but was still apparent and the movements of the reserpine-treated animals were somewhat erratic and labored. Perhaps the period of hyperactivity seen at the forty-eight hour interval following administration of the drug may in some manner be related to the prolonged

low level of serotonin in the brain, i.e. a form of "nonspecific stress response" to the biochemical lesion induced by reserpine.

3. The effect of methyldopa on the spontaneous activity of rats and the concentration of serotonin in the brain.

The effect of methyldopa on spontaneous activity in the rat is illustrated graphically in Figure 4 and is tabulated in Table VI. The depressant effect of the drug on this parameter was seen immediately and was much more rapid than that seen when reserpine was administered. This rapid depressant action is in excellent agreement with the findings reported by Smith (57) in his studies with this drug on mice. Smith (57) noted the return of activity to normal in mice eight hours after subcutaneous administration of methyldopa. That the activity in the animals in the present experiments did not recover to normal levels after the drug until twelve hours may be due to species difference, route of administration, or perhaps to potentiation of depression by the constituents of the vehicle. It may be seen from Figure 4 and Table III that the methyldopa vehicle caused a notable but transient depression of activity two hours after administration. This depressant action was found to be barely significantly different from normal ($0.02 < p < 0.05$). The depressant action of methyldopa on spontaneous activity (Figure 4, Table VI) at the two-hour interval when compared to the control group having received only the vehicle was highly significant ($p < .001$).

Methyldopa effectively lowered the concentration of serotonin in the rat brain (Figure 3, Table VII). This reduction in serotonin concentration was highly significant when compared with the concentration in normal animals ($p < .001$) at all time intervals examined until twelve hours after the administration of the drug. Animals receiving the control solution showed no change in brain serotonin concentration (Table VIII). Methyldopa has previously been shown by a number of workers to reduce the concentration of serotonin in the brain (37,57,58,63,88,89). It has also been shown that a reduction

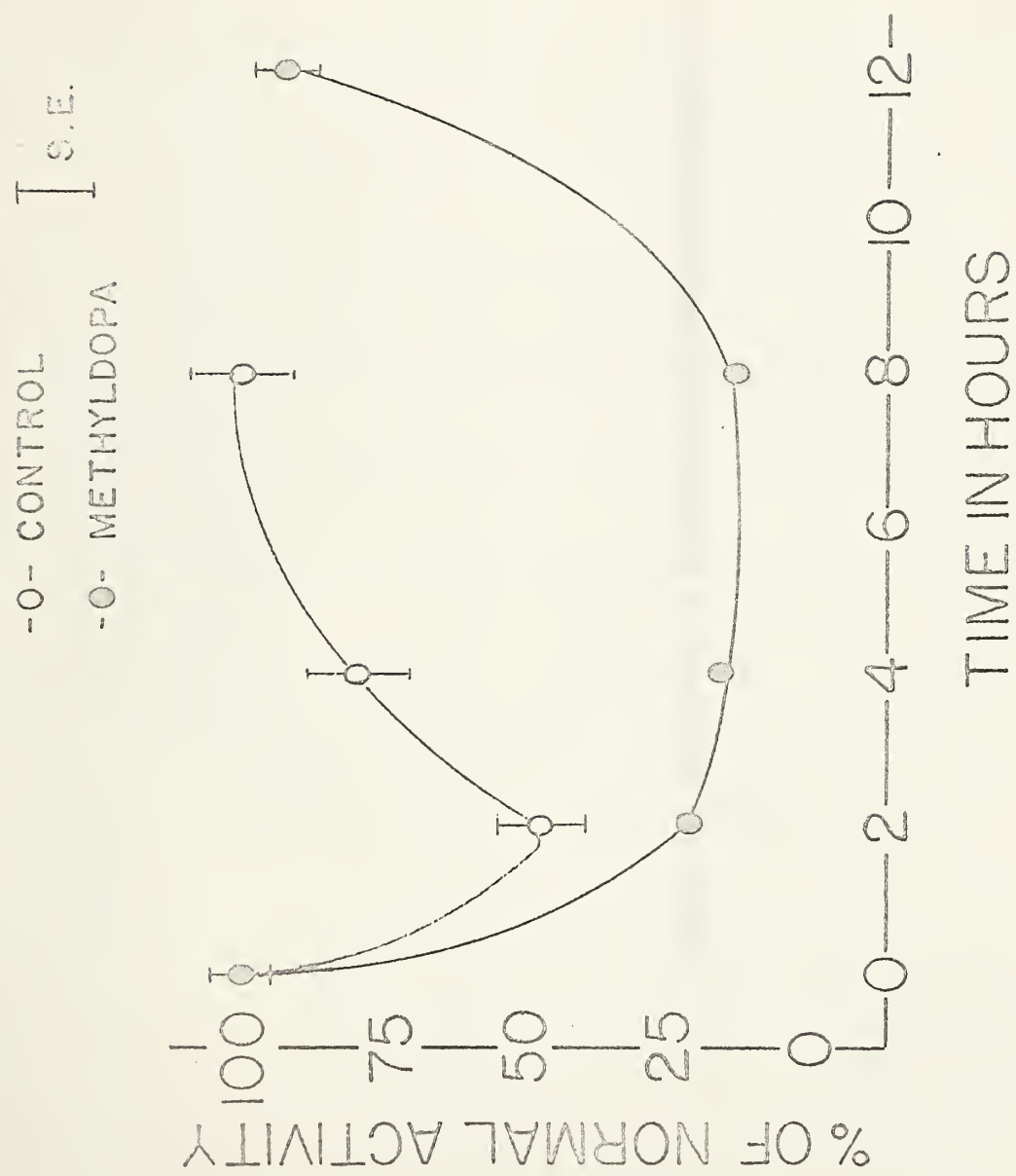


Figure 4: The Effect of Methyldopa on Spontaneous Activity of the Rat.

TABLE VI The Effect of Methyldopa (100 mg/kg, i.p) on Spontaneous Activity of the Rat

No.	Time After Drug Administration			
	2 Hours	4 Hours	8 Hours	12 Hours
1	6	8	8	34
2	8	3	3	21
3	10	8	14	36
4	7	7	9	30
5	4	4	3	22
6	8	6	10	35
7	7	15	2	45
8	9	9	0	47
9	8	7	4	37
10	10	4	8	36
11	12	6	5	33
12	6	8	10	45
13	8	8	7	32
14	7	5	6	28
15	10	4	6	34
16	4	4	5	40
Mean Counts/Min.	8	7	6	35
S.D.	0.7	3	3	7
S.E.	0.2	1	1	2
% S.E.	2	11	14	5
Mean Weight (g)	180	113	193	149

Normal activity: 38c/m (TABLE I)

TABLE VII The Effect of Methyldopa (100 mg/kg, i.p.) on Brain Serotonin in the Rat

(The figures in each column refer to the net fluorometer % fluorescence per gram fresh brain)

No.	Time After Drug Administration			
	2 Hours	4 Hours	8 Hours	12 Hours
1	0.41	0.46	0.62	0.74
2	0.58	0.51	0.63	0.77
3	0.60	0.62	0.59	0.83
4	0.66	0.58	0.60	0.74
5	0.58	0.48	0.71	0.82
6	0.56	0.52	0.58	0.83
7	0.59	0.41	0.61	0.79
8	0.64	0.50	0.74	0.81
9	0.70	0.49	0.59	0.65
10	0.55	0.51	0.58	0.80
11	0.60	0.48	0.54	0.73
12	0.59	0.52	0.63	0.87
13	0.66	0.53	0.61	0.91
14	0.58	0.45	0.70	0.73
15	0.60	0.54	0.57	0.87
16	0.57	0.49	0.58	0.85
Mean	0.59	0.51	0.62	0.80
S.D.	0.07	0.04	0.07	0.04
S.E.	0.02	0.01	0.02	0.01
%S.E.	3.0	2.2	2.9	1.4
Mean Weight (g)	180	193	193	149

Value for normal animals is 0.81 (TABLE I). This represents a concentration of serotonin in the brain of 0.54 ug/g.

TABLE VIII The Effect of Methyldopa Placebo (Controls) on Brain and Thyroid Serotonin in the Male Rat

(The figures in each column refer to the net fluorometer % fluorescence per gram fresh tissue)

No.	Time After Drug Administration					
	2 Hours		4 Hours		8 Hours	
	Brain	Thyroid	Brain	Thyroid	Brain	Thyroid
1	0.78		0.54		0.62	
2	0.84		0.82		0.81	
3	0.92		0.78		0.78	
4	0.64	13.2	0.98	14.0	0.74	12.7
5	0.66		0.69		0.88	
6	0.69		0.84		0.87	
7	0.86		0.82		0.81	
8	0.84	12.3	0.79	13.8	0.90	13.4
Mean	0.78	12.75	0.78	13.9	0.80	13.0
S. D.	.09		.12		.08	
S. E.	.04		.05		.03	
% S.E.	4.6		5.9		3.6	
Mean Weight (g)	201		190		190	

Value for normal animals is 0.81 (TABLE I). This represents a concentration of 0.54 ug/g serotonin in brain and 3.55 ug/g in the thyroid.

in serotonin concentration occurs in other tissues as well (57,87).

4. The effect of methyldopa pretreatment on the depressant action of reserpine on rat spontaneous activity and brain serotonin concentration.

When reserpine was administered to animals two hours after the administration of methyldopa the depressant effect of reserpine on spontaneous activity was reduced and recovery toward normal became apparent at about six hours (Figure 2, Table IX). The difference in activity of reserpinized animals pretreated with methyldopa and those given only reserpine at the eight-hour interval was highly significant ($p < .001$). Similarly, the difference between these groups at the twelve-hour interval following reserpine treatment was of equal statistical significance. The effect of methyldopa pretreatment on the depressant action of reserpine at the twenty-four hour interval is statistically indistinguishable ($0.2 < p < 0.3$) from that seen with reserpine alone.

It can therefore be stated that methyldopa pretreatment effectively increased the rate of recovery of spontaneous activity in animals treated with reserpine without shortening the total recovery time. That this phenomenon was due to a blocking action on the synthesis of free serotonin is not immediately apparent although this suggestion might be considered in view of the data in Figure 3 and Table X. These data illustrate that the initial serotonin releasing action of reserpine is temporarily but significantly impaired by methyldopa pretreatment. The statistical difference between the concentration of serotonin in the brain tissue of untreated rats and in rats given reserpine for a two hour period prior to decapitation for brain serotonin estimation was highly significant ($p < .001$). By the same token, the statistical difference between the concentration of serotonin in the brain two hours after administration of methyldopa and the concentration of serotonin in the brains of animals given reserpine for a two hour period after the initial pretreatment (2 hours) with methyldopa was not significant

TABLE IX

The Effect of 2-Hour Methyl dopa Pretreatment (100 mg/kg, i.p.) on Reserpine-induced (2 mg/kg, i.p.) Depression of Spontaneous Activity in the Male Rat

No.	Time After Reserpine				
	2 Hours	4 Hours	8 Hours	12 Hours	24 Hours
1	0	9	11	18	34
2	4	0	27	9	39
3	16	2	20	5	20
4	3	0	17	10	28
5	9	4	34	16	21
6	8	4	19	11	26
7	0	0	11	4	40
8	12	0	4	14	31
9	14	6	10	7	36
10	2	2	6	20	33
11	0	0	7	32	20
12	0	2	14	32	24
13	8	0	14	15	27
14	1	3	21	18	40
15	6	1	10	24	27
16	8	4	15	21	35
Mean activity	6	2	14	16	30
S. D.	5	3	8	8	7
S. E.	1	1	2	2	2
S. S.	17	30	13	13	6
Mean Weight (g)	140	153	148	170	154

Normal Activity: 38 c/m (TABLE I)

TABLE X

The Effect of 2-Hour Methylidopa Pretreatment (100 mg/kg) on Reserpine-induced (2 mg/kg) Release of Serotonin from the Male Rat Brain

(The figures in each column represent the net fluorometer % fluorescence reading per mg fresh thyroid tissue from four pairs of pooled glands in each experiment)

Expt. No.	Time After Reserpine					
	2 Hours	4 Hours	8 Hours	12 Hours	24 Hours	
1	.49	.32	.29	.39	.52	
2	.76	.25	.39	.38	.62	
3	.57	.36	.37	.34	.52	
4	.32	.32	.35	.34	.54	
5	.61	.40	.32	.40	.53	
6	.58	.27	.27	.41	.30	
7	.55	.38	.30	.31	.53	
8	.52	.30	.27	.32	.34	
Mean	.55	.32	.32	.36	.49	
S. D.	.11	.05	.04	.05	.10	
S. E.	.04	.02	.02	.02	.04	
C. I. E.	7.3	5.5	4.9	5	8.2	
Mean Weight (g)	140	153	148	170	154	

Value for normal % fluorescence/g fresh tissue is 0.81 (TABLE I), which represents a concentration of serotonin in the brain of 0.54 μ g/g.

($0.4 < p < 0.5$). This apparent inhibition of serotonin release was not reflected in a similar change in spontaneous activity as would be expected if serotonin release per se were the causative factor in the central depressant mechanism. If decarboxylase inhibition by methyldopa had a role in the effects seen, then, the resulting decrease in brain serotonin synthesis would have, according to Brodie's theory, resulted in increased spontaneous activity. This did not appear to occur. Following this initial delay in the effect of reserpine on brain serotonin release it can be seen (Figure 3, Table X) that the concentration of serotonin in brain tissue of animals given reserpine after methyldopa pretreatment did not decrease to the level seen when reserpine alone was administered. In addition, it is apparent that animals given methyldopa prior to reserpine showed a much more rapid return to normal in the concentration of brain serotonin. The serotonin concentration in the rat brain at the twenty-four hour interval with methyldopa pretreatment was significantly higher than in those animals receiving reserpine alone for the same time period ($p < .001$). Since there is an approximate correlation in the rate of recovery of spontaneous activity in animals pretreated with methyldopa prior to reserpine (Figure 2, Table IX) and their brain serotonin concentration (Figure 3, Table X) it might be speculated that at this time interval the inhibition of decarboxylase by methyldopa might have caused a decrease in the rate of biosynthesis of serotonin as originally anticipated in the experimental design of this investigation. However, since there was no correlation between brain serotonin concentration and spontaneous activity in animals given reserpine alone, attempts to associate a causal relationship between the degree of activity and brain serotonin concentration in the animals pretreated with methyldopa would not be valid. In addition, it might have been expected that decarboxylase inhibition would have resulted in a decreased brain serotonin concentration rather than the increase seen in these experiments.

Since the decreased concentration of serotonin in the brain of reserpinized animals pretreated with methyldopa increased at a more rapid rate than in animals given reserpine alone, it must be concluded that methyldopa acted in a manner similar to iproniazid (63), by partially inhibiting the serotonin releasing action of reserpine.

Experiments presently in progress in this laboratory indicate that serotonin release is not a causative factor in reserpine-induced depression. Rats given a second dose of reserpine twenty-four hours following the first injection, i.e. after recovery of spontaneous activity was approaching normal, were very heavily sedated for up to twenty-four hours. Analysis of the concentration of serotonin in the brains of these animals showed that this sedation was not accompanied by a decrease in the concentration of serotonin in the brain. The first dose of reserpine apparently caused maximal depletion of serotonin from the brain. Therefore, when the second dose was administered, the sedative effect of the drug could not be explained on the basis of brain serotonin release, since there was no change in the concentration of serotonin on the brain. A summary of the data in these experiments is given by the following: The average activity in fifteen animals 2 hours following the second dose of reserpine was 1 count/minute (range: 0 to 8). The brain serotonin concentration in 4 of these animals chosen at random was 0.28, 0.29, 0.30, 0.30 (expressed as percent fluorescence per gram fresh tissue).

II THE ROLE OF SEROTONIN IN THE THYROID DEPRESSANT ACTION OF RESERPINE

1. Studies on normal and adrenalectomized animals.

Experiments were performed in an attempt to study thyroidal I^{131} (iodide) uptake to give a measure of thyroid activity under normal conditions and after adrenalectomy. The data illustrated in Figure 5 and Table XI indicate that thyroidal I^{131} uptake is approximately linear for at least the first six hours following administration of the isotope in normal animals. The effect of extirpation of the adrenal glands three days prior to examination of thyroid function under this condition is illustrated in Figure 5 and Table XII. It will be noted that there was an apparent increase in thyroid function following adrenalectomy although the linearity of the I^{131} uptake "curve" in the six hour period studied was somewhat erratic.

2. The effect of reserpine on thyroidal I^{131} uptake in normal rats.

The influence of reserpine on thyroid activity in normal animals was studied under various conditions in an attempt to determine the mechanism involved in the reported antithyroid action of the drug. It can be seen in Figure 6 and Table XIII that reserpine depressed thyroidal I^{131} uptake under certain conditions whereas the administration of an appropriate volume of the vehicle was without significant effect ($0.4 < p < 0.5$). Administration of the drug in a dose of 0.2 mg/kg two hours before administration of radioiodine caused a 30% depression of I^{131} uptake at the four hour interval as compared to normal animals. The difference in thyroidal I^{131} uptake under the influence of this dose of the drug was statistically significant from the normal uptake ($p < .001$).

When a ten-fold increase in the dose of reserpine to 2.0mg/kg was made it was found that the antithyroid action was increased two-fold since thyroidal four hour I^{131} uptake was depressed approximately 60% at this dose level. This depressant action of the drug on thyroidal I^{131} uptake was highly significant when the data were compared with the normal four hour I^{131}

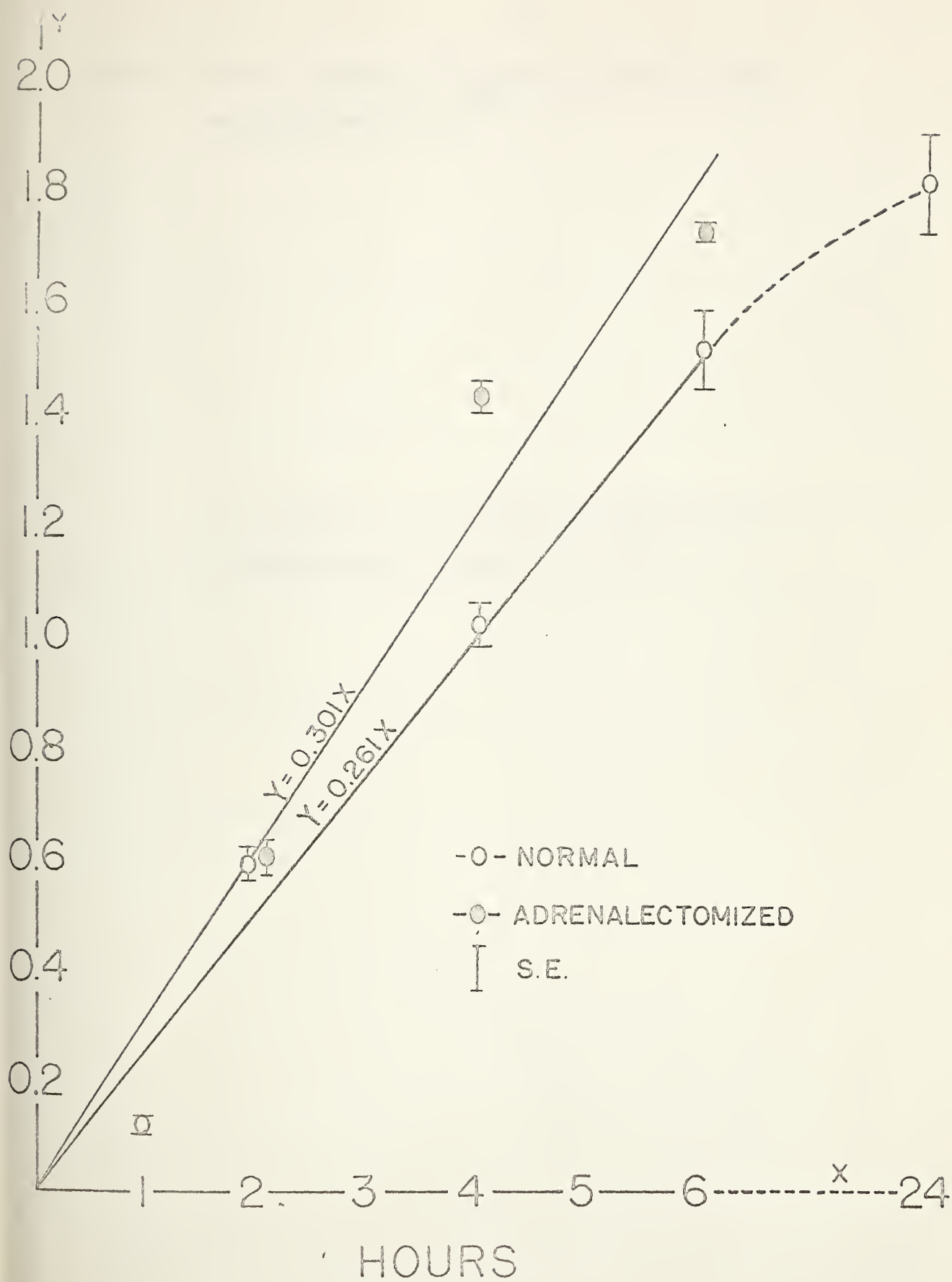


Figure 5: Rate of Thyroidal I^{131} Uptake in Normal and 3-Day Adrenalectomized Rats.

The equations for the calculated regression lines are expressed for the linear portions only.

TABLE XI Normal Rate of Thyroidal I¹³¹ Uptake in the Male Rat

(Percent Uptake of Administered Dose per mg Thyroid Tissue)

No.	1 Hour	2 Hours	4 Hours	6 Hours	24 Hours
1	0.118	0.463	0.998	1.56	1.75
2	0.109	0.675	0.933	1.17	1.65
3	0.108	0.735	0.937	1.63	1.81
4	0.137	0.468	1.11	2.01	1.22
5	0.149	0.520	0.824	1.76	1.98
6	0.121	0.470	0.996	1.30	1.90
7		0.613	1.02	1.45	1.87
8		0.544	1.01	1.80	1.78
9		0.650	0.853	1.16	1.73
10		0.498	1.21	1.47	1.92
11		0.715	1.00	1.44	2.42
12		0.695	0.975	1.46	
13			1.26		
14			1.20		
Mean Uptake	0.124	0.587	1.02	1.52	1.82
S. D.	.01	.10	.15	.23	.28
S. E.	.005	.03	.04	.07	.09
% S.E.	4.0	5.1	4.1	4.5	4.8
Mean Weight (g)	124	146	118	127	187

TABLE XII Normal Rate of Thyroidal I¹³¹ Uptake in the 3-day Adrenalectomized Male Rat
(Percent Uptake of Administered Dose per mg Thyroid Tissue)

No.	2 Hours	4 Hours	6 Hours
1	0.615	1.42	1.82
2	0.563	1.40	1.71
3	0.553	1.32	1.72
4	0.768	1.39	1.67
5	0.681	1.42	1.66
6	0.550	1.49	1.80
7	0.561	1.57	
8	0.419		
9	0.582		
10	0.671		
Mean Uptake	.596	1.43	1.73
S. D.	.09	.08	.03
S. E.	.03	.03	.01
% S.E.	5.1	2.4	0.8
Mean Weight	152	1.46	153

METHOD OF I-131 UPTAKE, HOURS

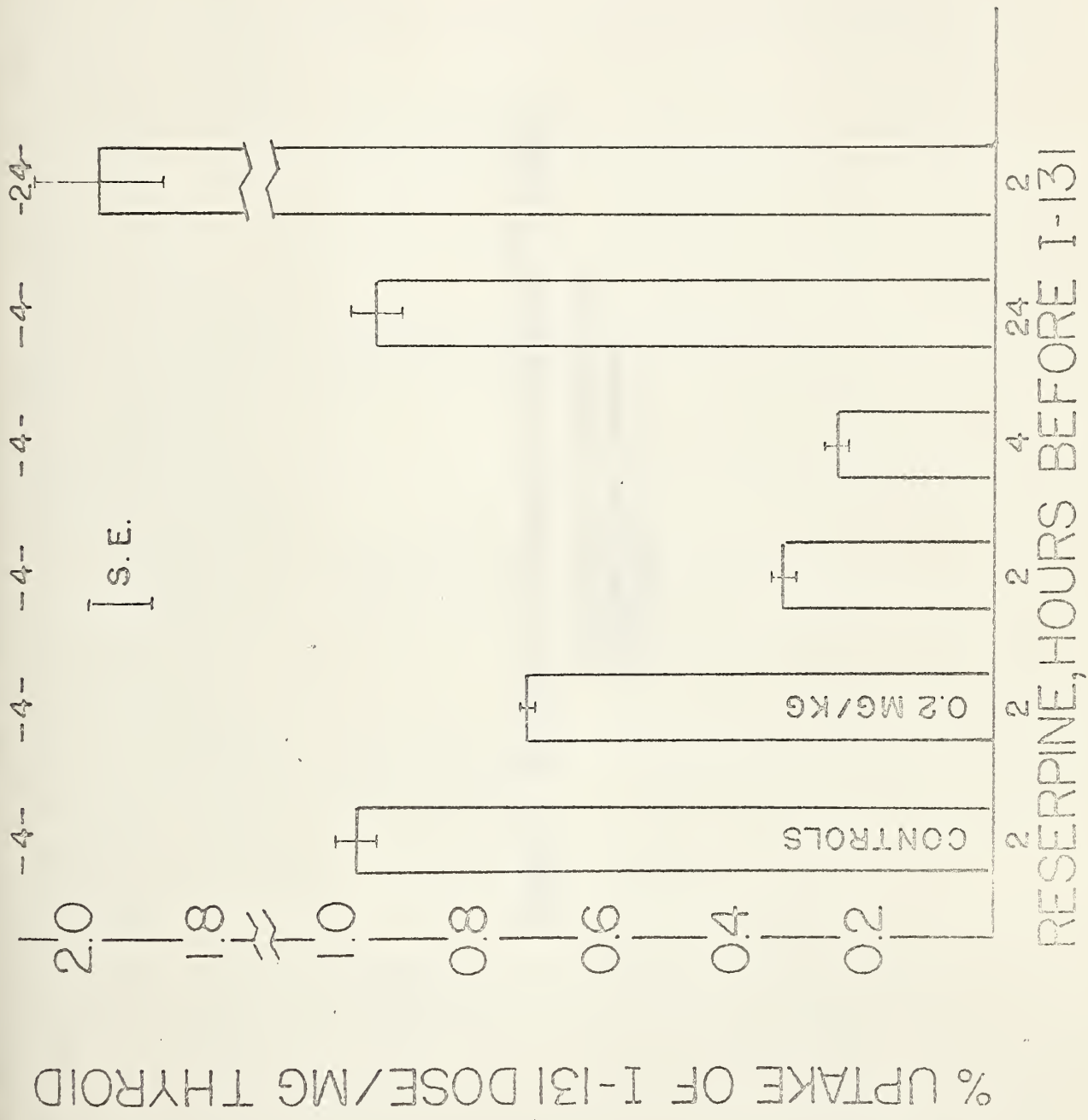


Figure 6: The Effect of Reserpine on Thyroidal I¹³¹ Uptake.

All dosages of reserpine are
2 mg/kg unless otherwise
indicated.

TABLE XIII The Effect of Reserpine on Thyroidal ^{131}I Uptake in the Male Rat

(Percent Uptake of Administered Dose/mg Thyroid Tissue)

No.	Dose and Duration of Pretreatment					
	Controls, 2 Hours	0.2 mg/kg, 2 Hours	2.0 mg/kg, 2 Hours	2.0 mg/kg, 4 Hours	2.0 mg/kg, 24 Hours	2.0 mg/kg, 2 Hr., I-131, 24 Hours
1	0.978	0.700	0.381	0.219	1.055	1.59
2	0.962	0.709	0.292	0.283	1.190	2.41
3	0.921	0.728	0.286	0.238	1.042	1.91
4	0.919	0.710	0.271	0.255	0.908	1.95
5	1.13	0.725	0.300	0.174	0.949	1.92
6	0.926	0.731	0.359	0.197	0.935	2.29
7		0.663	0.333	0.225	0.804	2.08
8		0.739	0.340	0.288	0.829	1.53
9					0.833	
10					0.847	
Mean Uptake	0.973	0.713	0.320	.235	0.939	1.96
S. D.	.06	.03	.04	.04	0.12	0.28
S. E.	.03	.01	.02	.02	.04	0.11
% S.E.	2.8	1.6	4.7	8.5	4.3	5.5
Mean Weight (g)	115	145	103	128	182	201

uptake ($p < .001$). When the drug was administered at this dose level four hours prior to administration of the radioiodine it was found to depress uptake of the iodine by the thyroid gland to 25% of normal uptake ($p < .001$).

When reserpine in a dose of 2 mg/kg was administered twenty-four hours prior to administration of the radioiodine the depression of thyroidal four hour uptake of the isotope was reduced by only 8% from the normal uptake (Figure 6, Table XIII). This slight depressant action of reserpine did not alter the uptake significantly when compared with the data seen in normal animals ($.05 < p < 0.1$). The effect of pretreatment of the animals with reserpine (2 mg/kg) for two hours followed by an iodine uptake period of twenty-four hours resulted in an apparent increase in thyroidal ^{131}I uptake which did not differ significantly from the normal twenty-four hour uptake ($0.3 < p < 0.4$).

These results support the previous claims of several investigators that reserpine depresses thyroid function (38,39,41,45,46,47) and suggests that this antithyroid effect may be seen only during the first few hours following administration of the drug. Since the antithyroid action of reserpine was to some extent dependent on the dose employed and on the duration of thyroid uptake and pretreatment it is not surprising that some reports have appeared in the literature claiming that the drug does not affect thyroid function (40,42,43,45,90,91,92).

An adequate study of the in vivo effect of reserpine on the fate of inorganic iodide has not yet appeared in the literature. Such a study might provide an explanation for the apparent lack of change in thyroidal ^{131}I uptake under the influence of reserpine. Perhaps the ^{131}I was rerouted into a certain fraction and then released after the uptake mechanism was re-established. This postulate is based on the speculation that since the twenty-four hour uptake was unchanged after

reserpine, excretion of the "temporarily untrappable" iodine was not likely to have occurred.

In the in vitro system of Mayer et al (44) reserpine ($1.4 \times 10^{-4}M$) was seen to depress the incorporation of I^{131} into various organic fractions isolated from incubating calf thyroid slices with little effect on the uptake of inorganic iodide into the slices. Mayer and his colleagues concluded that this antithyroid action of reserpine was the consequence of a direct action of the drug on the iodide trapping mechanism. A report by Galton and Ingbar (93) that reserpine and serotonin depressed in vitro the degradation of thyroxine suggested that the antithyroid action of reserpine might in some way be related to its effect on serotonin binding in tissues by making serotonin available to the thyroid gland via the blood stream. Since serotonin has been found in the thyroid gland (77,78) it was felt that an examination of the effect of reserpine and some related drugs on thyroidal serotonin concentration would provide evidence that the antithyroid action of reserpine reported above and by previous workers might be intimately related to the effects of the drug on serotonin storage in the gland.

3. The effect of reserpine, methyldopa, and guanethidine on the concentration of serotonin in the thyroid gland of the rat and on thyroidal I^{131} uptake.

Examination of the effects of reserpine on the concentration of serotonin in the thyroid gland indicated that the drug caused a pronounced and rapid decrease of thyroidal serotonin concentration within two hours after administration of the drug (Figure 7, Table XIV). This depleting action on thyroidal serotonin was seen up to at least sixty hours following administration of the drug (Table XIV). Methyldopa (Table XV, Figure 7) was also effective in reducing the concentration of serotonin in the thyroid gland. Its effect on serotonin was of much shorter duration than that seen with reserpine and a recovery of thyroidal serotonin concentration

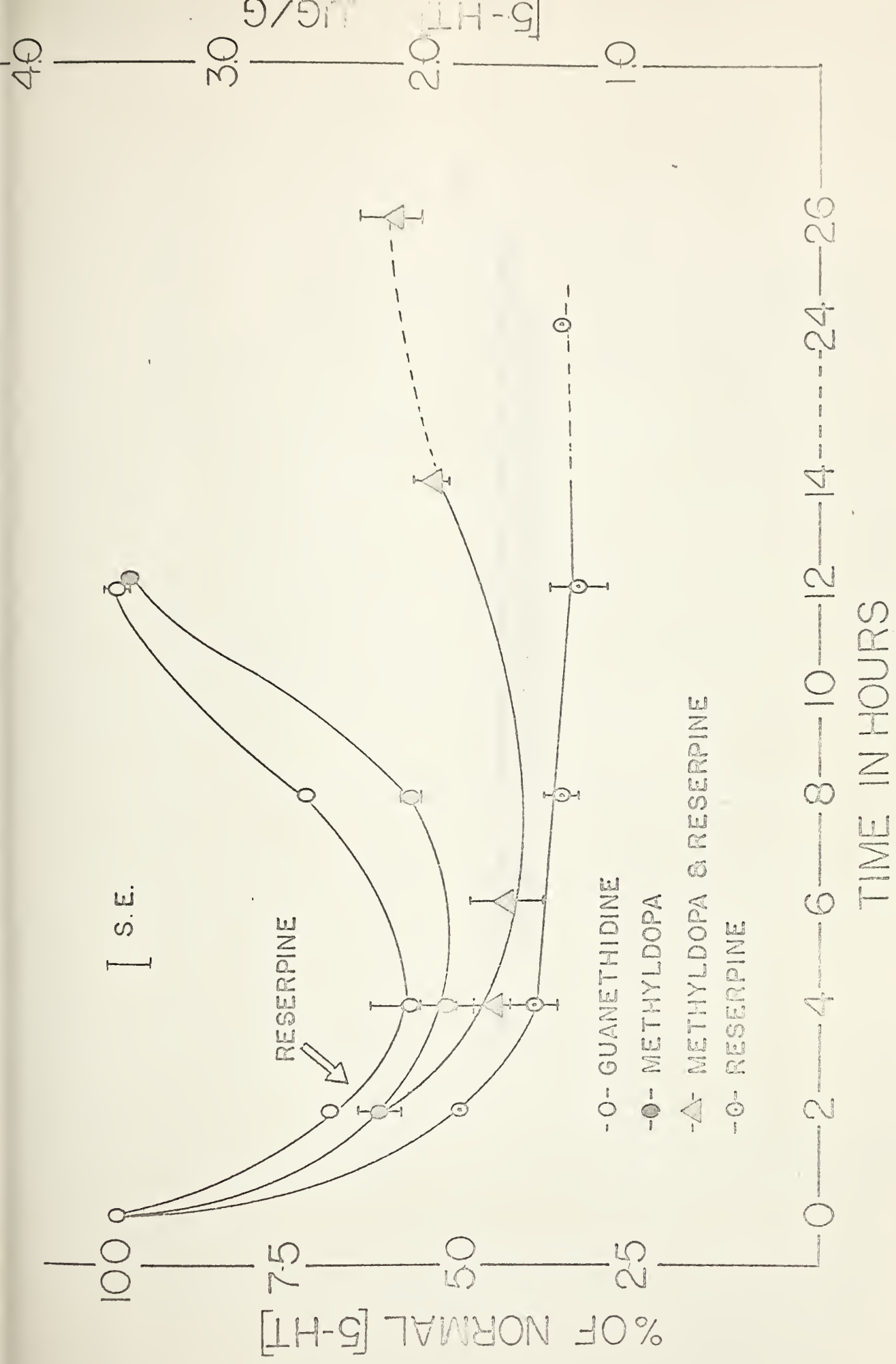


Figure 7: The Effect of Guanethidine, Methyldopa, Reserpine, and Methyldopa
Followed by Reserpine on Thyroidal Serotonin Concentration.

TABLE XIV The Effect of Reserpine (2 mg/kg, i.p.) on Thyroid Serotonin Level in the Rat

(The figures in each column represent the net fluorometer % fluorescence reading per mg fresh thyroid tissue from four pairs of pooled glands in each experiment)

Expt. No.	Time After Drug Administration					
	2 Hours	4 Hours	8 Hours	12 Hours	24 Hours	60 Hours
1	6.90	4.80	5.0	3.9	5.7	4.9
2	6.40	4.61	5.7	3.7	5.4	5.9
3	6.97	5.42	4.2	5.9	4.0	
4	7.24	6.80	4.5	1.9	4.7	
Mean	6.9	5.4	4.8	4.6	4.9	5.4
S.D.	0.3	0.9	0.6	0.9	0.7	-
S.E.	0.2	0.5	0.3	0.5	0.4	-
% S.E.	2.5	9.1	6.8	11.0	7.7	
Mean Weight (g)	170	168	181	179	182	152

Value for normal % fluorescence in untreated animals is 13.4 (TABLE I), which corresponds to 3.55 ug serotonin/g fresh thyroid tissue.

TABLE XV The Effect of Methyldopa (100 mg/kg, i.p.) on Thyroid Level of Serotonin in the Rat

(The figures in each column represent the net fluorometer % fluorescence reading per mg fresh thyroid tissue from four pairs of pooled glands in each experiment)

Expt. No.	Time After Drug Administration			
	2 Hours	4 Hours	8 Hours	12 Hours
1	8.0	8.3	8.0	12.8
2	7.8	6.8	8.1	13.7
3	8.2	5.9	7.5	13.3
4	9.6	7.4	7.7	13.0
Mean	8.4	7.1	7.8	13.2
S.D.	0.7	0.9	0.4	0.3
S.E.	0.4	0.5	0.2	0.2
%S.E.	4.9	7.2	2.7	1.5
Mean Weight (g)	180	113	193	149

Value for normal animals is 13.4 (TABLE I). This corresponds to 3.55 ug/g serotonin in the thyroid.

was seen within twelve hours. Guanethidine was examined for an effect on thyroid serotonin since it is similar to reserpine in that it has been reported to release norepinephrine from various peripheral sites (94,95,96), and reportedly releases serotonin from the intestine (97). Guanethidine was seen to act on the thyroid in a manner more typical of methyldopa as it produced a marked reduction in the concentration of serotonin in the thyroid gland which was no longer apparent at twelve hours (Table XVI, Figure 7). This effect of guanethidine on the concentration of serotonin in the thyroid gland is likely due to a reserpine-like release mechanism since the drug apparently has no effect on the decarboxylating enzyme responsible for the formation of serotonin from its immediate precursor (98).

When methyldopa and guanethidine were examined for effects on thyroidal I^{131} uptake they also were found, in addition to reserpine, to have a depressant effect on this parameter. The data in Figure 8 and Table XVII clearly illustrate that both drugs were effective inhibitors of I^{131} uptake under the conditions of these experiments. The effects of guanethidine on thyroidal I^{131} uptake were highly significant from normal ($p < .001$). The depressant action of methyldopa on this parameter was different from its control groups at a high degree of significance ($p < .001$).

It is therefore quite apparent that all of the drugs examined had a depleting effect on thyroidal serotonin and simultaneously produced a significant depression of thyroidal I^{131} uptake. It was not possible on the basis of this evidence alone to conclude that thyroidal serotonin release was a priori in the effect of these agents on thyroid function. Experiments were therefore undertaken to provide further evidence to support or disprove the hypothesis that serotonin release was related to the effects seen on the thyroid with these drugs.

TABLE XVI The Effect of Guanethidine Sulfate (5 mg/kg, i.p.) on Thyroid Level of Serotonin in the Rat

(The figures in each column represent the net fluorometer % fluorescence reading per mg fresh thyroid tissue from four pairs of pooled glands in each experiment)

Expt. No.	Time After Drug Administration				
	2 Hours	4 Hours	8 Hours	12 Hours	
1	8.9	9.5	9.8	13.2	
2	9.5	6.3	10.3	13.9	
3	8.9	8.3	9.7	13.5	
4	9.9	7.1	9.8	12.9	
Mean	9.3	7.8	9.9	13.4	
S.D.	0.12	1.2	0.22	0.35	
S.E.	0.07	0.7	0.13	0.20	
% S.E.	0.7	9.0	1.3	1.5	
Mean Weight (g)	170	170	150	153	

Value for normal animals is 13.4 (TABLE I). This corresponds to 3.55 ug/g serotonin in the thyroid.

PERIOD OF I-131 UPTAKE, HOURS



Figure 8: The Effect of Guanethidine and Methyldopa on Thyroidal I^{131} Uptake.

TABLE XVII The Effect of Guanethidine and Methyldopa on Thyroidal I¹³¹ Uptake in the Male Rat
(Percent Uptake of Administered Dose/mg Thyroid Tissue)

DURATION OF PRETREATMENT

Guanethidine 5 mg/kg	Methyldopa 100 mg/kg			
	Control		Drug	
	2 Hours	4 Hours	2 Hours	4 Hours

DURATION OF I-131 UPTAKE

No.	2 Hours		4 Hours		4 Hours			
1	0.236	0.350	0.654	0.631	0.496	0.420		
2	0.210	0.291	0.677	0.616	0.504	0.403		
3	0.209	0.320	0.694	0.561	0.445	0.459		
4	0.391	0.496	0.628	0.609	0.494	0.447		
5	0.284	0.423	0.656	0.620	0.548	0.414		
6	0.284	0.453	0.696	0.632	0.497	0.489		
7	0.318	0.554		0.689	0.555	0.474		
8	0.256	0.538		0.634	0.564	0.498		
9		0.416			0.472	0.481		
Mean Uptake	0.274	.427	0.668	0.624	0.508	0.454		
S. D.	.06	.09	.03	.03	.04	.03		
S. E.	.02	.03	.01	.01	.01	.01		
% S.E.	7.9	7.26	2.0	1.8	2.8	2.3		
Mean Weight (g)	156	155	135	138	115	111		

4. Blockade of the thyroid depressant action of reserpine by dibenzyline and methyldopa.

The class of adrenergic blocking agents known as beta-haloalkylamines has been known for some time to be effective serotonin antagonists in addition to possessing sympatholytic properties (99,100). Phenxybenzamine (Dibenzyline is the hydrochloride) perhaps is the best known of these compounds and has been used almost routinely whenever selective blockade of alpha-adrenergic receptors (101) is desirable in a given pharmacological experiment. Johnson and Sellers (102) presented evidence that the drug could adequately block the stimulating effect of adrenomedullary epinephrine on oxygen consumption of rats which was induced by reserpine.

In the present experiments dibenzyline was administered two hours prior to the injection of reserpine and methyldopa in an attempt to block the action of serotonin released by the drugs. It can be seen that such pre-treatment effectively blocked the depressant action of both drugs on thyroidal I^{131} uptake (Figure 9, Table XVIII). Dibenzyline itself had no effect on thyroidal I^{131} uptake when compared to its vehicle ($0.6 < p < 0.7$). That the blockade of the thyroid depressant action of reserpine was not due to the constituents of the debenzyline vehicle is shown in Figure 9 and Table XVIII ($0.5 < p < 0.6$, compared to the effect of reserpine alone).

The inhibition of the depressant action of reserpine and methyldopa on thyroidal I^{131} uptake by dibenzyline can probably be attributed to blockade of the action of the serotonin released by the drugs. It may be argued that peripheral catecholamine release might play a role in the thyroid depressant action of these drugs since epinephrine is known to depress thyroid function (103). However, subsequent experiments on adrenalectomized animals which will be described later ruled out this possi-

PERIOD OF I-131 UPTAKE: 4 HOURS

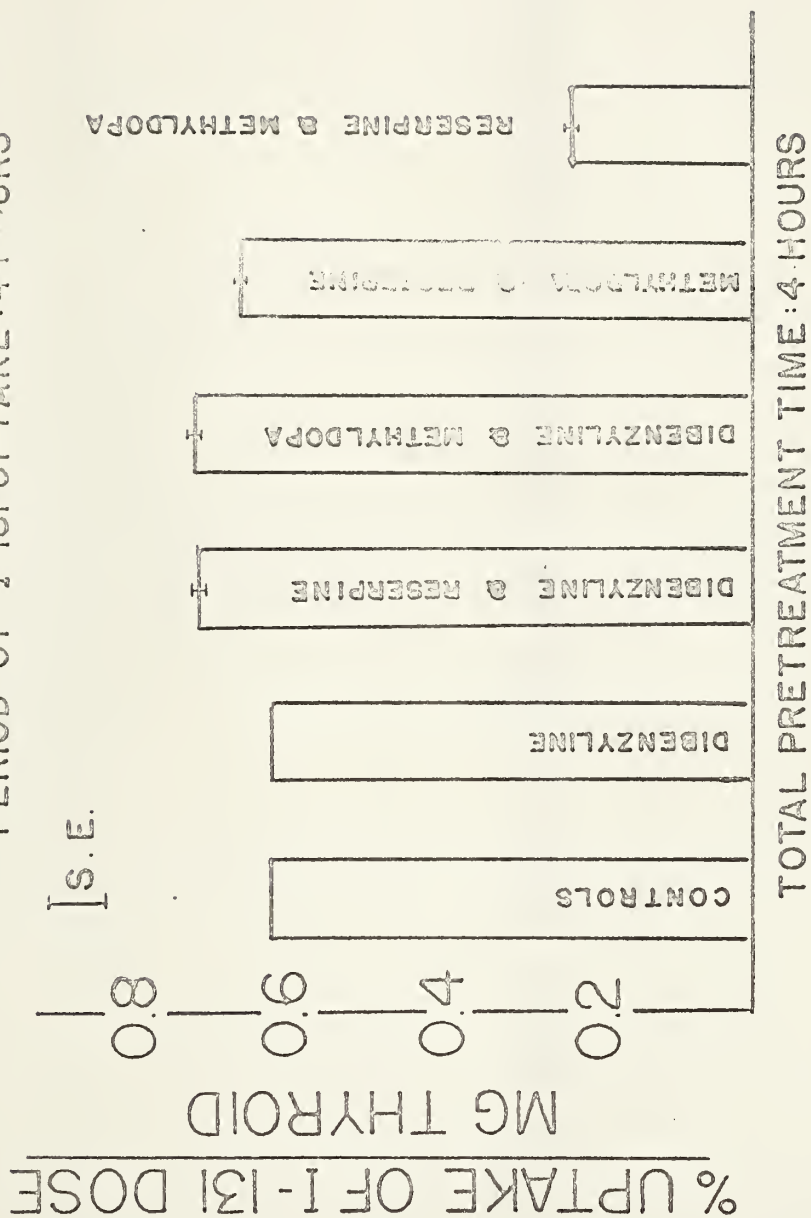


Figure 9: Blockade of the Thyroid Depressant Action of Reserpine and Methyldopa.

TABLE XVIII

Blockade of the Depressant Action of Reserpine (2 mg/kg, i.p.) on Thyroidal 4-Hour I^{131} Uptake in the Male Rat by Dibenzylamine (2 mg/kg) and Methyldopa (100 mg/kg) Pretreatment.

No.	Time (Hours) Prior to I^{131} Administration					
	Dibenzylamine 4	Vehicle 4 Reserp. 2	Dibenzylamine 4 Reserpine 2	Dibenzylamine 4 Methyldopa 2	Methyldopa 4 Reserpine 2	Reserpine 4 Methyldopa 2
1	.597	0.324	0.729	0.674	.732	0.233
2	.603	0.318	0.735	0.727	.667	0.202
3	.628	0.334	0.695	0.703	.668	0.233
4	.647	0.319	0.649	0.728	.630	0.201
5	.592	0.329	0.758	0.740	.655	0.206
6	.619	0.361	0.662	0.706	.610	0.217
7	.642	0.337	0.670	0.749	.621	0.253
8	.599	0.319	0.741	0.720	.693	0.257
9					.677	
10					.687	
11					.673	
12					.614	
Mean Uptake	.616	0.330	0.705	0.718	.661	0.225
S. D.	.02	.02	.04	.03	.03	.02
S. E.	.006	.006	.01	.01	.008	.009
S. S.E.	0.91	1.8	1.8	1.7	1.2	3.9
Mean Weight (g)	145	150	144	140	111	136

bility.

When methyldopa was administered prior to reserpine the depressant action of the latter drug on thyroid function was significantly inhibited ($p < .001$) (Table XVIII, Figure 9) and the thyroid depressant action of either drug was not seen. This paradoxical situation was somewhat puzzling since it was expected that the antithyroid action of methyldopa at least, would be noticeable in these experiments. It was at first felt that a situation similar to that seen in the brain of animals pretreated with methyldopa and then given reserpine (Figure 3, Table X) was occurring in the thyroid gland, i.e. that methyldopa delayed the release of serotonin from the gland when reserpine was administered. However, when the thyroid glands of animals treated with methyldopa prior to reserpine were examined for their serotonin concentration it was found that such an effect did not occur (Figure 7, Table XIX). The gradual return to normal of thyroidal serotonin concentration was seen in this tissue as was noted previously in the brain.

Consideration of all the data available on this subject, both in these experiments and in the literature, suggested that serotonin release was related to the effects of reserpine on thyroid function. It was speculated that the inhibiting action of methyldopa on reserpine-induced thyroid depression was manifested in the brain by inhibition of serotonin release since the drug was without apparent effect on the action of reserpine on thyroidal serotonin storage during the period of I^{131} uptake studied. It therefore appeared reasonable to investigate the possibility that the thyroid depressant action of reserpine was primarily a centrally mediated

TABLE XIX

The Effect of 2-Hour Methylodopa Pretreatment (100 mg/kg) on Reserpine-induced (2 mg/kg) Release of Serotonin from the Male Rat Thyroid Gland

(The figures in each column represent the net fluorometer % fluorescence reading per mg fresh thyroid tissue from four pairs of pooled glands in each experiment)

Expt. No.	Time After Reserpine			
	2 Hours	4 Hours	12 Hours	24 Hours
1	4.5	6.2	7.7	6.2
2	6.3	5.3	8.1	9.1
3	8.2	4.7	6.8	8.3
4	5.8	7.8	7.1	8.6
Mean	6.2	6.0	7.4	8.05
S. D.	1.3	1.2	0.6	1.1
S. E.	0.8	0.7	0.3	0.6
% S.E.	12.3	11.3	4.6	7.9
Mean Weight (g)	140	153	170	154

Value for normal animals is 13.4 (TABLE 1). This corresponds to 3.55 μ g/g serotonin in the thyroid.

phenomenon.

5. The effect of adrenalectomy and hypophysectomy on the thyroid depressant action of reserpine.

When the effect of reserpine on thyroid function was studied in hypophysectomized animals (two days after surgery) it was found that the drug was without effect on thyroidal I^{131} uptake in two separate experiments (Figure 10, Table XX). This finding suggested an involvement of the pituitary-adrenal axis on the effect of reserpine on the thyroid gland. Experiments with adrenalectomized rats (three days after surgery) showed at the four hour period of I^{131} uptake that the depressant effect of reserpine on thyroid function was greater than the comparable effects seen in normal animals (Figure 6), (Figure 10, Table XXI). This enhanced effect of reserpine was not seen in those animals in which a two hour period was allowed for I^{131} uptake.

It is apparent that the action of reserpine on the thyroid gland is probably a collection of events related to the release of serotonin from the brain. Westermann et al (51) have indicated that the hypersecretion of ACTH by reserpine is directly related to the release of serotonin from the brain of animals receiving the drug. Since ACTH is known to depress TSH (thyroid stimulating hormone) secretion from the anterior pituitary (104) it would not seem unreasonable to expect the hypersecretion of ACTH to reduce the output of TSH from the anterior pituitary. Further evidence that serotonin release is involved in the process is indicated by a report (105) that dibenamine, a beta-haloalkylamine closely related to dibenzylamine, blocked the initial ACTH releasing action of reserpine. In this case, the blockade of the antithyroid action of reserpine may be considered to be at the level of interference with the access of the agonist, serotonin, to its receptive sites. That the antagonism of reserpine-induced thyroid depression seen in these experiments was effected at two distinct levels

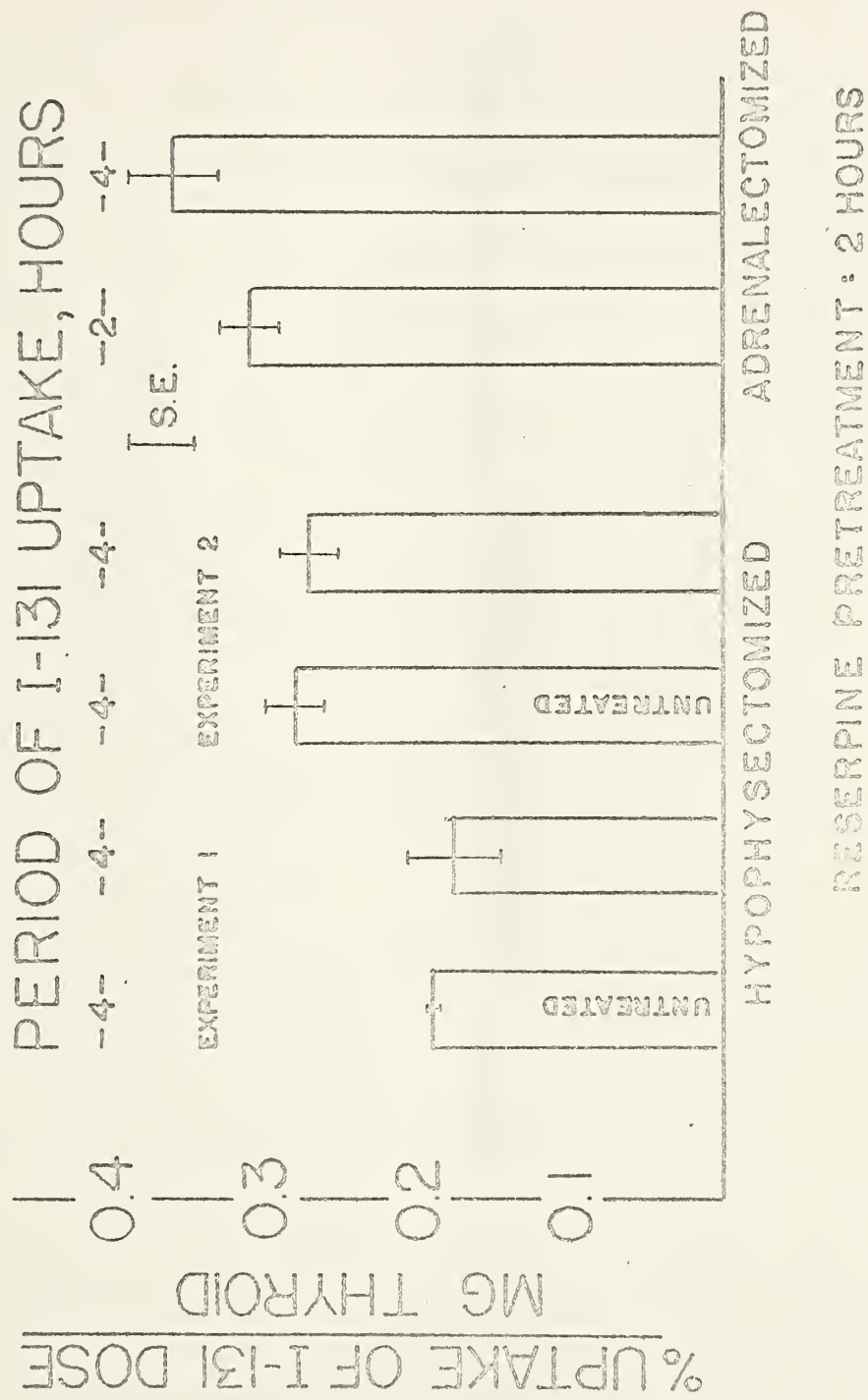


Figure 10: The Effect of Hypophysectomy and Adrenalectomy on the Thyroid
Depressant Action of Reserpine.

TABLE XX

The Effect of 2-Day Hypophysectomy on the Depressant Action of Reserpine
(2 mg/kg, i.v.) on Thyroidal 4-Hour I-131 Uptake in the Male Rat

(Percent Uptake of Administered Dose/ing Thyroid Tissue)

No.	Experiment 1		Experiment 2	
	Normal	Reserpine	Normal	Reserpine
1	.196	.143	.243	.291
2	.175	.241	.364	.287
3	.182	.194	.263	.243
4	.170	.126	.288	.241
5			.255	.327
Mean Uptake	.180	0.176	0.283	0.278
S. D.	.01	.05	.04	.03
S. E.	.006	.03	.02	.02
S. D.	3.3	14.8	7.1	5.4
Mean Weight (g)	145	141	138	141

Reserpine was administered 2 Hours prior to the I¹³¹

TABLE XXI The Effect of 3-Day Adrenalectomy on the Depressant Action of Reserpine
(2 mg/kg, i.p.) on Thyroidal I-131 Uptake in the Male Rat

(Percent Uptake of Administered I¹³¹ Dose per mg Thyroid Tissue)

Duration of Pretreatment with Reserpine		
	2 Hours	2 Hours
Duration of Thyroidal I ¹³¹ Uptake		
No.	2 Hours	4 Hours
1	.285	.546
2	.394	.306
3	.361	.226
4	.332	.403
5	.293	.377
6	.202	.373
7	.414	.336
8	.297	.351
9		.280
10		.423
Mean Uptake	0.322	0.362
S. D.	.06	.08
S. E.	.02	.03
% S.E.	7.6	7.6
Mean Weight (g)	152	168

is illustrated in the effects seen with methyldopa pretreatment. This antagonism can be said to result from inhibition of release of the agonist, serotonin from its storage sites thereby preventing its accessibility to specific sites functioning in the regulation of ACTH secretion. Consistent with the view that methyldopa acts to prevent the brain serotonin releasing action of reserpine is the report by Necina (106) that the drug inhibited the depleting effect of reserpine on adrenal cholesterol and ascorbic acid, changes which are usually indicative of pituitary hyperactivity.

GENERAL DISCUSSION

The results of these experiments tend to indicate that the previous literature pointing to a role of serotonin release in the central depressant action of reserpine must either be re-evaluated or rejected. In view of the findings presented herein, it is difficult to associate reserpine-induced central sedation with release of serotonin from the brain. It may be that the depletion of brain serotonin by reserpine has a secondary role in the effect of the drug. Preliminary experiments in this laboratory have shown that the second of two successive doses of reserpine given twenty-four hours apart results in almost immediate sedation as compared with the relatively gradual onset of sedation seen with the drug in the first instance. The more rapid onset in depression seen with the second dose may be due to the fact that the low level of serotonin in the brain at the time of the second dose facilitated the effect of the drug. Perhaps the initial release of serotonin represents a threshold which is necessary for the reserpine to overcome. In this sense, once the amine levels have become depleted the effect of the drug is much more rapidly induced.

It is quite apparent from the experimental evidence available that Brodie's hypothesis of the role of serotonin in the central depressant action of reserpine is an incorrect interpretation of the process. Support for this conclusion has been recently presented by Gal et al (107). They have shown that the administration of reserpine to rats made serotonin deficient by maintenance on a tryptophan-free diet resulted in a sedative effect due to the drug with no change in the already low (30% of normal) level of serotonin.

Serotonin release may have an important place when other parameters

of the pharmacologic actions of reserpine are considered. A relationship between brain serotonin release after reserpine administration and thyroid function has been described. The effect of reserpine on thyroid function may be postulated in the following manner (Figure 11).

The first step in the mechanism involves the release of bound serotonin to give free serotonin (I), a process which is inhibited temporarily by methyldopa (and also by monoamine oxidase inhibitors (MAOI)). Associated with this step is the hypothalamic depression seen with reserpine (108, 109, 110) which may also be a function of the serotonin concentration. Free serotonin then could pass into the pituitary gland via the hypothalamico-hypophyseal portal system (111) to bring about the hypersecretion of ACTH (III). This effect can be blocked by dibenzylamine. The hypersecretion of ACTH then depresses the secretion of TSH (IV). Depression of the anterior hypothalamus also occurs which may influence this process (V). In the latter regard, it has been shown that the anterior hypothalamus regulates thyrotropin secretion to some extent (112). When the adrenal glands are removed (VI) the reserpine induced, serotonin mediated, hypersecretion of ACTH is increased since the surgical removal of the pituitary-adrenal negative feedback mechanism allows ACTH hypersecretion to proceed unchecked. The net result of all of the contributing factors is a decrease in hypophyseal TSH secretion. Other workers have suggested a relationship between the action of reserpine and TSH secretion but have presented no mechanism of action or direct evidence to support it (113, 114, 115, 116).

There is adequate proof for the existence of each individual step in this scheme. That the collection of these events represents the process by which reserpine depresses thyroid function must remain a hypothesis until these suggestions can be verified.

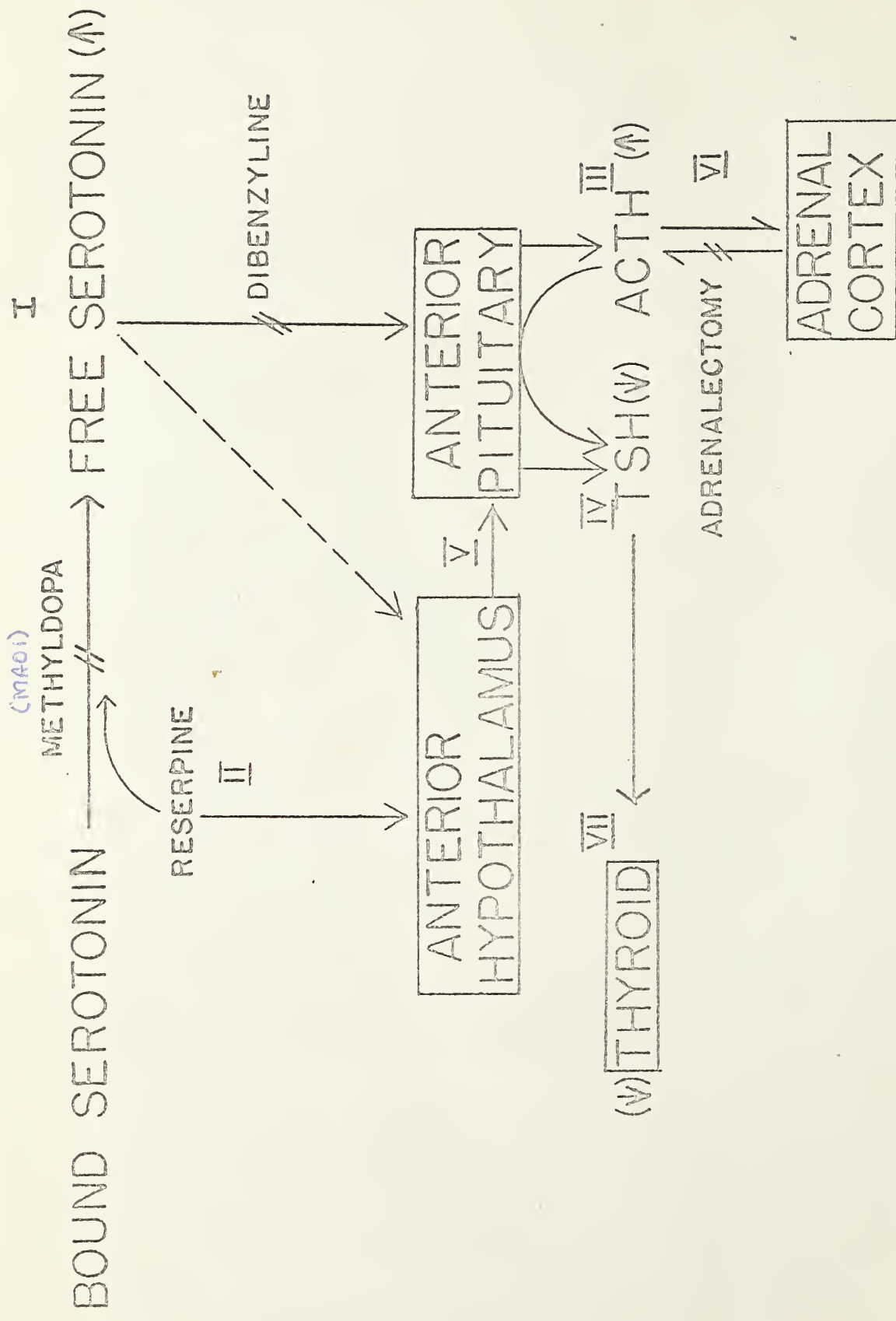


Figure 11: A postulated mechanism explaining the thyroid depressant action of reserpine.

the results of the study are presented in Table 1. The results show that the proposed method is more accurate than the existing methods.

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CONCLUSIONS

The proposed method is more accurate than the existing methods. The results of the study are presented in Table 1. The results show that the proposed method is more accurate than the existing methods.

1. Serotonin release appears to have no direct relationship to the central depressant action of reserpine.
2. The depressant action of reserpine on thyroid function is probably mediated by serotonin release within the brain.
3. It is postulated that this release of serotonin initiates the hypersecretion of corticotropin (ACTH) by the pituitary which results in decreased thyrotropin (TSH) output by this gland. The decreased secretion of thyrotropin is the underlying cause of the depressed thyroid function.

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APPENDIX

EVALUATION OF THE METHOD
OF SEROTONIN ESTIMATION

Analytical work in general is meaningless unless some indication of the reliability of a procedure is presented with the data thus obtained. In this project the method for estimation of serotonin was evaluated with the following objectives:

1. Agreement in identity with the chemically pure substance
2. Recovery
3. Interference in fluorescence analysis by extraneous substances

1. AGREEMENT IN IDENTITY WITH THE CHEMICALLY PURE SUBSTANCE

The criterion for agreement in identity with the pure substance was the absorption and fluorescent maxima obtained with tissue extracts and spectra obtained with the pure substance passed through the complete analytical procedure.

Agreement in absorption and fluorescence maxima occurred when examined on the cathode ray oscillograph attached to the spectrophotofluorometer.

This procedure has been an acceptable criterion by many workers and has been verified by suitable chromatographic techniques by several investigators using this solvent system for extraction of amines from tissues. In other words it would appear that serotonin was the only substance being measured.

2. RECOVERY OF SEROTONIN FROM TISSUE HOMOGENATES

In each experiment two brains were pooled and homogenized in 0.01N HCl as before, except that a total volume of 5ml of homogenate was obtained. This homogenate was then halved to yield two 2.5ml homogenates. To one homogenate was added 0.5ml of the acid (0.01N HCl) to the other was added 0.5ml of the acid containing a certain amount of serotonin. This resulted in two identical homogenates except that one contained the added amine. In all cases, the extraction procedure was followed as before from this point.

Percent Recovery Calculation:

The final extracts of each homogenate pair were composed of:

1. Endogenous amine and added amine

2. Endogenous amine

Thus, subtraction of the fluorescence obtained in extract 2 from that obtained in extract 1 gave the net fluorescence due to the amount of added volume present in the final stage of the procedure.

After correction for aliquot removal, this figure was then compared with the fluorescence obtained from the serotonin standards.

Aliquot Correction Factors For Serotonin: $\frac{15^a}{10} \times \frac{3.2^b}{1} = 4.80$

a) to correct for transfer of $\frac{10}{15}$ of the butanol initial to the heptane-HCl mixture.

b) to correct for removal of $\frac{1}{3.2}$ ml of the final acid extract (which increases from 3.0 ml to 3.2 ml due to water in the butanol).

The results of the recovery experiments are presented on the following page.

3. INTERFERENCE IN FLUORESCENCE ANALYSIS BY EXTRANEEOUS SUBSTANCES

Norepinephrine, epinephrine, and dopamine did not interfere in the analysis for serotonin when added to standard solutions in "physiological" quantities.

Recovery of Serotonin

Net % Fluorescence				% Recovery	
Amount	Pure Standard	Standard Extract ^a	Tissue Extract ^b	Standard Extract	Tissue Extract
0.5 µg	2.15	0.41 x 4.8 = 1.97	-	$\frac{1.95 \times 100}{2.13} = 91.6\%$	-
	2.10	0.40 x 4.8 = 1.92			
1.0 µg	4.35	0.84 x 4.8 = 4.03	0.81 x 4.8 = 3.89	$\frac{3.98 \times 100}{4.30} = 92.6\%$	$\frac{3.87}{4.30} = 90.0\%$
	4.25	0.82 x 4.8 = 3.94	0.80 x 4.8 = 3.84		
1.5 µg	6.50	1.25 x 4.8 = 6.00	-	$\frac{6.12 \times 100}{6.65} = 92.0\%$	-
	6.80	1.30 x 4.8 = 6.24	-		
2.0 µg	-	-	1.60 x 4.8 = 7.68	-	$\frac{7.56 \times 100}{8.76} = 87\%$
			1.55 x 4.8 = 7.44		

a) Fluorometer reading corrected for aliquot removal

b) Net fluorescence from added serotonin

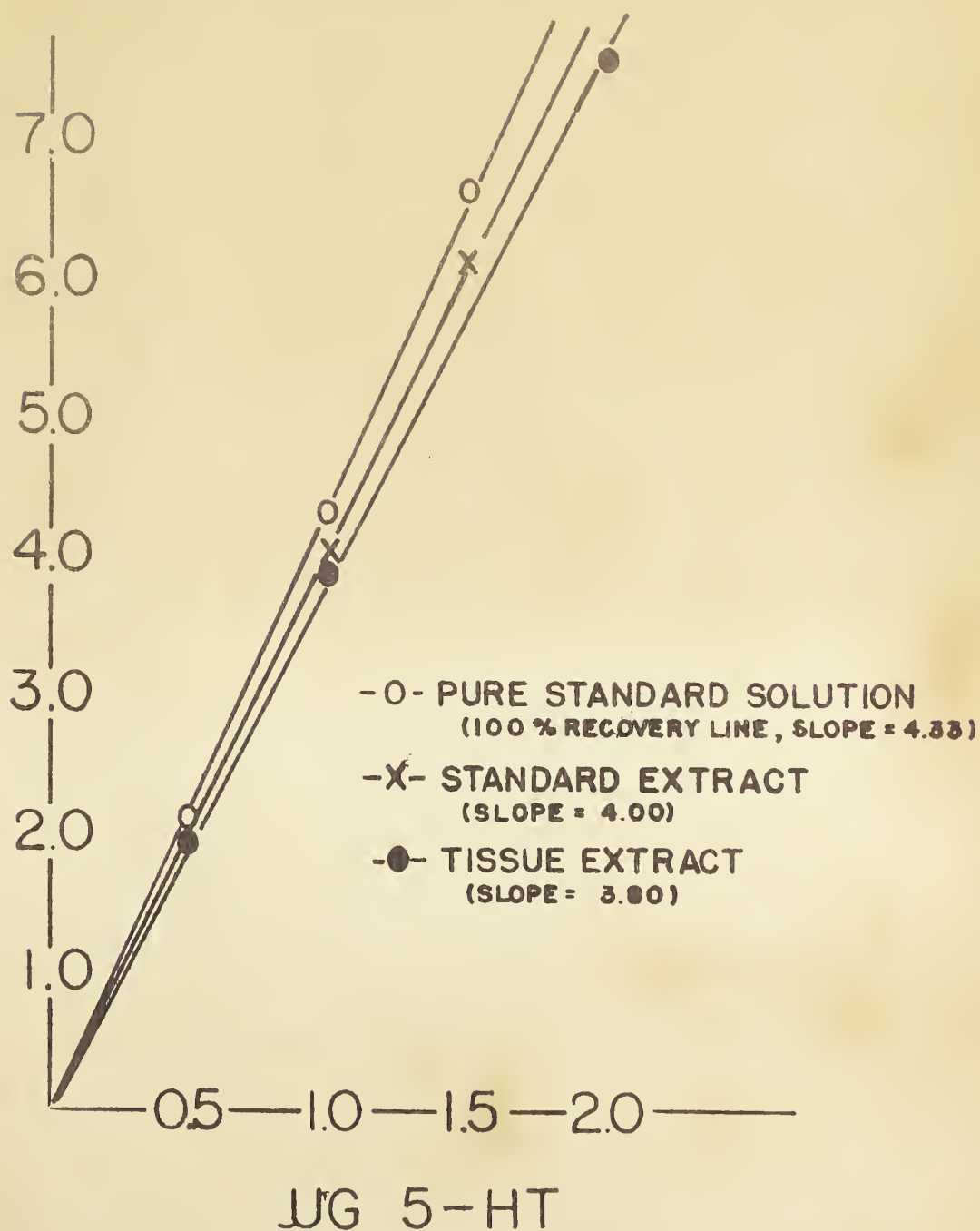
c) Extrapolated from graph (see following page)

Summary:

Mean Recovery of Standard Serotonin (Slope of Graph) $\frac{100 \times 4.00}{4.33} = 92.5\%$

Mean Recovery of Serotonin Added to Homogenate (Slope of Graph) $\frac{3.8 \times 100}{4.0} = 88.5\%$

RECOVERY OF 5-HT



B29812